DEVELOPMENT OF RAPID BIOASSESSMENT PROTOCOLS FOR OKLAHOMA UTILIZING CHARACTERISTICS OF THE DIATOM COMMUNITY

OKLAHOMA CONSERVATION COMMISSION

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INTRODUCTION

The use of biological communities as indicators of water quality has changed frequently as the understanding of the interactions between water quality and the integrity of biological communities has evolved (Dixit et al., 1992). Historically, most water quality programs have been directed towards the collection and analysis of chemical samples to determine the effects of obvious point sources of pollution such as industrial or municipal wastewater plant discharges (Hughes et al., 1990; Round, 1991). This approach is limited in that it yields very little data concerning 'normal' or non-polluted conditions in lakes and streams and without this information, assessment of the degree of impairment or management of aquatic resources is very difficult (Karr et al., 1986; Hughes and Larsen, 1988). The most effective way to address the limitations imposed by the reliance on chemical analyses in water quality assessments is to assess the health or integrity of the biological communities (Hughes et al., 1990; Round, 1991). The assumption made when using a biological community in the assessment of water quality is that the structure of a biological community directly reflects the chemical and physical properties of the water (Patrick, 1977; Round, 1991; Karr et al., 1986; Hughes et al., 1990). As a general rule, more organisms will be adapted to live in streams of relatively pristine or undisturbed condition than those whose quality has been altered and this will be reflected in a more diverse community (Round, 1991). In other words, the majority of organisms which cannot tolerate stress will be replaced by the relative few which can (Carins, 1973). It is also recognized that particular organisms are adapted to specific chemical conditions so that their presence (or absence) can be used to make conclusions concerning water quality (Kolkwitz and Marsson, 1908; Kolbe, 1932; Lange-Bertalot, 1979; Descy, 1979; Hughes et al., 1990; Round, 1991).

CHAPTER 2

SUBJECT BACKGROUND

A. Components of the Aquatic Community

Three components of the aquatic biological community have been commonly used in the assessment of water quality; invertebrates, fish, and algae (periphyton), although protozoans and bacteria have been used in areas of extreme organic pollution (Fjerdingstad, 1964). Each group has particular qualities which make it desirable as a tool in the assessment of water quality and each has limitations.

The most important factors relevant to the use of a particular component in water quality assessment can be summarized as follows (Dixit et al., 1992; Plafkin et al., 1989):

1. **Ease of sample collection**
2. **Response to water quality conditions**
3. **Number of taxa**
4. **Number of organisms**
5. **Habitat limitation**
6. **Complexity of identification**
7. **Geographical comparability**
8. **Distribution within habitats**
9. **Presence of quantitative methods**

The use of each of these three groups of aquatic organisms is discussed in detail in the "EPA Rapid Bioassessment Protocols for Use in Streams and Rivers", (Plafkin et al., 1989). Protocols similar to those already in existence for fish and benthic macroinvertebrates have yet to be developed for the algal community; therefore, the advantages and disadvantages of this group will be discussed briefly.

Next to the bacteria and protozoa, the algal community contains the largest number of organisms and the greatest taxonomic diversity in the aquatic environment. This community serves as the basis of the aquatic food chain, derives most of its nutrition from dissolved chemicals in the water (Patrick, 1973), and has important effects on water chemistry such as the production of oxygen (Meier and Dilks, 1984). The relationship of the algal community to the above

listed factors follows:

1. **Ease of sample collection.** Very easy to sample qualitatively; however, quantitative sampling is more time-consuming (requires two site visits per collection) and not precise (high coefficient of variation among samples).

2. **Response to water quality conditions.** Algae respond rapidly to water quality conditions and some taxa are recognized as indicators of specific water quality conditions; however, some groups vary in sensitivity to toxic substances (Selby et al., 1985; Millemann et al., 1984; Blanck, 1985; Stanislawska-Swiatkowska and Ranke-Rybicka, 1976).

3. **Number of taxa.** The algal community, particularly the periphyton component (diatoms), contains a very high number of taxa.

4. **Number of organisms.** This group of organisms contains the highest number of organisms among the three groups. The best group to use for statistical analysis of results.

5. **Habitat limitation.** Of the three groups discussed, benthic diatoms are the least affected by habitat limitation.

6. **Complexity of identification.** Most periphyton forms, particularly diatoms, can be easily identified to the genus level; however, identification to the species level requires considerable experience, extensive sample preparation, and a compound microscope.

7. **Geographical comparability.** Algae are very comparable in terms of their distribution across wide areas, including globally for most major genera and species.

8. **Distribution.** Although algae fill many different niches in the aquatic environment, the most common indicator group (diatoms) are more restricted.

9. **Quantitation.** Counting algae is not difficult but does require a compound microscope. Biomass measurement can be expensive and time consuming.

B. Rapid Bioassessment Protocols (RBPs)

Traditionally biological assessments have consisted of either the development of inventory lists or, less often, some determination of the structure and function of the various components of the aquatic community (Cairns, 1973; Weber, 1973, Hendrickson, 1977); however, there has been little in the way of systematic and consistent analysis. In recognition of both the problems

associated with biological monitoring and the importance of their place in stream assessments, the EPA has encouraged the development of new and more accessible biological assessment criteria. The goal has been the development of systems that are faster, simpler, produce quantifiable results, and provide assessments of equal (or superior) value to traditional methods. The RBP is a method for assessing the integrity of the biological communities based upon differences in community measures (metrics) between sample and reference sites (Plafkin et al., 1989, Yoder, 1989).

The use of reference sites assesses differences in the biological community which are due to water quality differences across a geographically similar area and provides information as to the minimally obtainable taxonomic diversity which could be expected within a geographical region (ecoregion).

C. Ecoregions

The "ecoregion" concept was developed by Omernik (1987) as means to divide the United States into regions which have a high degree of ecological similarity. The U.S. was divided into seventy-six ecoregions, nine of which occur in Oklahoma (Figure 1). It has been suggested that these ecoregions may be too large in some cases which might result in considerable variation in natural biological communities across their range. In these case it is recommended that these ecoregions be sub-divided into appropriate sub-units (Hughes et al., 1990). This would appear to be a likely necessity in Oklahoma.

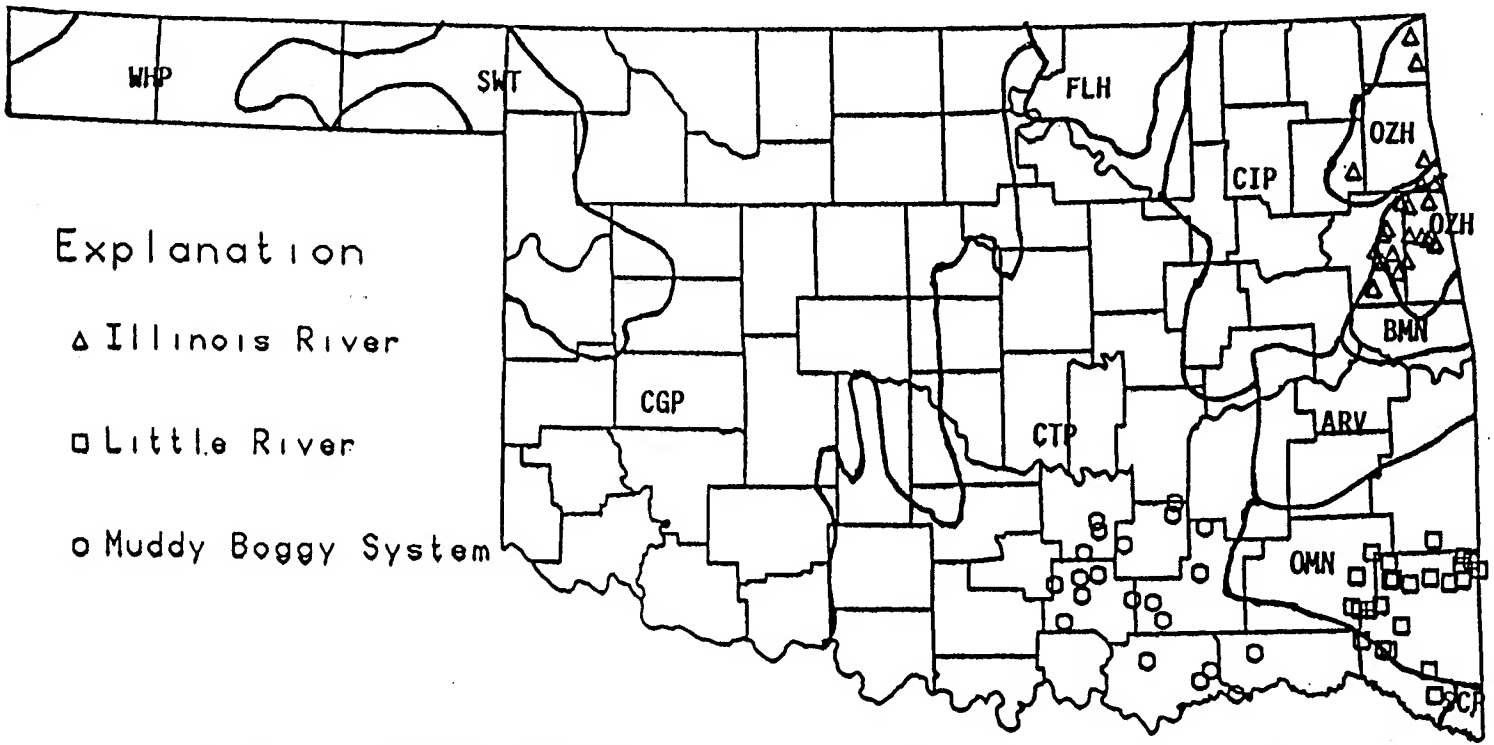

FIGURE 1. OKLAHOMA ECOREGIONS. SWT = Southwestern Tablelands, FLH = Flint Hills, WHP = Western High Plains, CGP = Central Great Plains, OZH = Ozark Highlands, CIP = Central Irregular Plains, BMN = Boston Mountains, ARV = Arkansas Valley, OMN = Ouachita Mountains, SCP = South Central Plains, CTP = Central Texas Plateau

The ecoregion approach separates areas which are ecologically and climatologically equivalent so that waterbodies within each ecoregion can be compared. This is opposed to the traditional method of basing reference conditions or standards on a wider politically-based area, such as a state. The chemical quality of natural waterbodies within an ecoregion should be similar; therefore, disturbances can be measured by referring to their relative effects on "natural" conditions rather than comparison to a numerical standard or conditions in geologically dissimilar areas. The uses and limitations of ecoregions are discussed in detail by Hughes and Larsen (1988) and Hughes et al. (1990).

D. Nomenclature of Algae in the Lotic Environment

Although the term periphyton is most commonly used in reference to the benthic algal community, this is not a truly accurate term as it infers that this community consists solely of autotrophic (phyton) organisms. The german term "aufwuchs", which can be loosely translated as "outgrowth", also has been widely used in the past but has decreased in popularity. These and other terms are inaccurate in that the biological growth on aquatic substrata contains a number of different taxonomic groups including protozoa, fungi, bacteria, mosses, algae, and others. The term periphyton is intended to refer only to autotrophic organisms, and in most cases only the algae, (and often only the diatoms); however, this term is used when referring to most community biomass measurements, which include all taxonomic groups present. This nomenclature dilemma is well reviewed by Weitzel (1979).

Perhaps the most accurate and descriptive terms would be "algal benthos", "phytobenthos", or "benthic algal community" as proposed by Hutchinson (1975); however, none of these terms term are widely accepted. Despite its shortcomings, periphyton remains as the most commonly used term to describe the benthic algal community; however, for the purpose of accuracy, the term epiphytobenthos (EPB) will be used in this document to describe the entire benthic algal community while the diatom component will be specifically referred to as such.

The epiphytobenthos can be broken down into the numerous groups of algae that make up this community, ranging from filamentous green algae and blue-green algae (cyanobacteria), to diatoms and others. Although many of these different groups can be found as the dominant algae under particular circumstances, the diatoms are generally the most common group both in terms of numbers, variety, and biomass (Weitzel, 1979; Douglass, 1958) and may make up greater than 95% of the EPB community (Keithan et al., 1988).

Diatom taxonomy is based entirely upon visual characteristics of the cell wall, unlike many other algae where sexual stages must be present for identification. In addition, most periphytic diatoms

are essentially unicellular, which facilitates enumeration over other cell arrangements, such as filaments. The RBPs developed in this document will focus entirely on the characteristics of the diatom community.

E. Sensitivity of Diatoms to Environmental Conditions

The principle goal of bioassessment is the identification and characterization of areas where degradation of water quality has occurred. Although all groups of aquatic organisms have a range of response to environmental conditions, diatoms have been shown to have a narrow range of optimal conditions and tolerances, respond quickly to changing environmental conditions, and exist in communities which are usually taxonomically diverse (Dixit et al., 1992), all of which are factors which make diatoms particularly suitable as indicators of environmental quality.

The response of the diatom community to various physical/chemical factors such as nutrients, toxins, light, temperature, competition, grazing pressure, and velocity has been well researched. The purpose of this document is to develop protocols for classifying streams according to the condition of the diatom community (degree of impairment) relative to that of reference stream(s)/conditions.

Assessment protocols do not take into account those factors which may be responsible for a level of impairment, other than habitat limitations. Streams are classified into one of the following categories: non-impaired, slightly impaired, moderately impaired, or severely impaired based solely on the make-up of the biological community. The goal of rapid bioassessment is the classification of streams so that efforts to identify and ameliorate the effects of those factors which are causing impairment can be focused on the areas of greatest need. With this in mind, there will be only a brief discussion of those factors which may be influencing the streams sampled during this study.

CHAPTER 3

HISTORY OF DIATOMS AS ENVIRONMENTAL INDICATORS

Diatoms have long been used as indicators of water quality conditions although to a lesser extent than other members of the aquatic community. Historically, there have been two approaches to using diatoms as indicators of environmental conditions; however, different approaches have been recently introduced. The first approach utilized the "indicator species" concept whereby particular species of known water quality preference (tolerance) were used to indicate water quality conditions. The second traditional approach has been more mathematical where the number and distribution of species present have been manipulated through various formulae to calculate a single numerical value. Newer systems such as the Hilsenhoff Botic Index (HBI) (Hilsenhoff, 1987) for benthic macroinvertebrates and the Index of Biotic Integrity (IBI) (Karr et al., 1986) for fish have sought to assess biological communities through the integration of multiple factors and are essentially a combination of the two historical approaches with other measures. Each of these approaches and their advantages and disadvantages will be reviewed in detail in the following discussion.

A. Indicator Organisms

The most widely recognized system of diatom classification was created by Kolkwitz and Marsson (1908). Their "saprobien" system was based on the classification of streams into various groups based upon the breakdown of introduced organic material. This system was based upon the theory that there were zones of organic decomposition within streams and that particular species, including diatoms, would be associated with a particular zone. The work of Kolkwitz and Marsson have been modified several times (Patrick, 1973; Weitzel, 1979; Fjerdingstad, 1964) and is still in use; however, these systems are not particularly useful in the classification of streams which do not receive point source additions of concentrated nutrients or organic wastes.

In order to address both the 'indicator value' of organisms and the numbers of an indicator species present, Chutter (1972) includes both a "quality value" and a numerical estimation to arrive at an importance value for benthic macroinvertebrate species. A similar approach is taken by Descy (1979) and Lange-Bertalot (1979) with diatoms, both of whom strongly advocate the use of indicator species. Other works (Gotoh; 1986a, 1986b) have also shown the usefulness of a combination of the "indicator species" concept in combination with numerical criteria. Ruth Patrick has done extensive work exploring the association between particular diatoms and water quality conditions and has found that the

indicator species concept is invalid without consideration of the relative numbers of species present. Patrick et al. (1949) classified streams as healthy, semi-healthy, polluted, or very polluted based upon the association of particular diatom communities with water quality conditions. Patrick (1973) states that there are a few species, which when found to be the dominant organism at a site, can be used to indicate a particular type of pollution or water quality zone, but that the degree of pollution cannot be quantified on this basis. In a later paper Patrick (1984) states that it is the relative abundance of certain associations of species, rather than the dominance by one species which should be used as water quality indicators. Associations of diatom species at a higher taxonomic level (family) have been used by Stockner (1971) to classify lakes, so in some instances it may not be necessary to identify to the species level to obtain water quality information.

B. Community Structure Indices

Because of the mis-use of the "indicator species" concept in the past, its limitations, and the reluctance of biologists to use information which would not be considered statistically significant in the traditional sense, a more mathematical approach to community analysis has been widely used. The rationale underlying community structure indices lies in the theory that high quality environments have more diverse and evenly distributed biological communities (Patrick and Roberts, 1979). The reason being that many species are not capable of adjusting to the environmental stress caused by pollution. This results in a reduction in the total number of species present and an increase in the number of specimens of those species which can survive the stress (Cairns, 1973). Healthy streams are represented by a relatively large number of species, with approximately equal distributions, while polluted streams are characterized by a shift in species composition to high numbers of a relatively few species. Additionally, a taxonomic shift takes place from the more taxonomically diverse diatom-dominated community to one dominated by green algae, or in extreme cases, blue-green (cyanobacteria) algae (Patrick, 1973). The many diversity indices which have been developed are an attempt to quantify these properties.

Boyle et al., (1990) reviewed nine of the most commonly used diversity indices and found that they varied greatly in their response to changes in the taxonomic make-up of fictitious communities. Some were more responsive to changes in the number of taxa, while others responded more to changes in the numbers of organisms. Washington (1984) found that only three of eighteen indices (diversity, biotic, similarity) were valuable in assessing water quality. Additional problems have been pointed out by Patrick (1977) who showed that very different communities would generate similar diversity index values. It has also been shown

that communities with very low numbers of individual species can yield a very high diversity index value and that low diversity can occur in the absence of pollution (Descy, 1979). According to Boyle et al. (1990) diversity indices should be judged based upon the following four factors:

1. Value of the index at the initial (undisturbed) condition of the community.

2. Sensitivity to community change.

3. Stability of the index response to reduction in density and species richness.

4. Consistency of response to patterns of community change.

Other indices, such as richness, dominance, evenness, and redundancy, which generate different information have been developed; however, these involve different mathematical manipulations of the same numbers with many of the same inherent problems. Redundancy is high when many of the same species are encountered in a sample; therefore, a higher value indicates poorer water quality. This is more or less the inverse of most diversity indices. Richness deals with both the number of taxa as well as their relative distribution, although Boyle et al. (1990) equate the number of species present with richness, whereas species diversity indices are focused more directly on distribution without regard to the number of taxa. In order to circumvent the problems with individual indices it has been suggested by Boyle et al. (1990) that more than one index be used in each case to assure that representative results are obtained.

C. Biotic Indices

Biotic indices have been developed to circumvent the problems associated with strict reliance on indicator species and/or community structure indices. Biotic indices are based on rigorous sampling and analysis methodologies and the assessment of the association (correlation) between physico-chemical conditions and biological integrity (Karr et al., 1986). For the most part they do not rely on the collection of additional data or increased levels of taxonomic or numerical analysis, but rather they integrate the concepts of indicator species, species associations, and measures of community structure. According to Patrick (1973) '.. it is important to use a variety of parameters in diagnosing the quality of water by the use of algae. One should consider the structure of a community, that is, the numbers of species, their relative abundance, the kinds of species, and the total biomass present. Using various combinations of these parameters, one can define the

effects of various types of pollution such as organic load, toxic materials, suspended solids, and temperature effects.' This is essentially a description of a biotic index.

The fish and benthic macroinvertebrate RBPs utilize several biotic indices, the most widely recognized of which are the HBI and IBI, although a similar index for benthic macroinvertebrates was developed by Chutter (1972). These indices take into account the factors suggested by Patrick, as well as others, and integrate them into a single analysis. The Index of Biotic Integrity as developed by Karr et al. (1986) incorporates information concerning twelve characteristics of the fish community including species richness, species composition, number of darter species, number of sucker species, number of intolerant species, number of intolerant species, trophic guilds, and health of individual fish.

It has been suggested that the use of indicator species, without regard to relative or absolute abundance can lead to erroneous conclusions. One of the most important papers which addresses the concept of utilizing diatoms as estimators of water quality was published by Descy (1979). In this study a system of rating diatoms was developed whereby the sensitivity to pollution of various species was determined as well as the ecological amplitude of the response. Diatoms were rated from one to five for sensitivity and 1 to 3 for amplitude of response or "indicating value". These numbers were arrived at by counting diatoms from various sites on the River Meuse and combining the results with chemical data utilizing correspondence factor analysis. When comparing this method with others, Descy (1979) found that there were poor correlations between these results and those achieved by using the saprobic index or diversity indices. He also found poor correlations between other methods and the chemical quality of the water in the area of study.

Lange-Bertalot (1979) uses a similar approach in classifying streams. He has taken the large body of chemical and biological information that exists on the Rhine River and has divided the diatom community into three groups: 1) those which are most tolerant to pollution, 2) those less tolerant and 3) those which cannot tolerate water conditions below (or above) some critical level. Streams are classified by the percent of the community which is made up of individual in the three groups. For example, if the majority of the diatoms found at a particular site belong to group 1, then the stream would be classified as relatively un-polluted. Lange-Bertalot has categorized approximately 100 of the most abundant and frequently found species into these three groups, and has observed that most of these species are relatively cosmopolitan in nature. He also found that of these 100 species, 50 make up as much as 99% of the population in most cases.

Schoeman (1979) working in South Africa used Lange-Bertalot's approach and found it to be consistent with the chemical analysis of the water and adaptable to South African conditions. This further illustrates the usefulness of the cosmopolitan characteristics of the diatom community and indicates that a similar approach could be used in Oklahoma. Because of these results, it can be concluded that a community can be defined by those organisms which constitute the majority of the population and water quality estimations can, therefore, be based upon this association. Under this type of system, it is not necessary to count thousands of organisms, but merely to identify those which dominate the community. It is anticipated that this concept will figure heavily in the development of rapid bioassessment protocols.

CHAPTER 4

PROJECT BACKGROUND

This research is being conducted through field studies at the Oklahoma Conservation Commission (OCC) funded under state programs and federal grants. OCC is involved in a number of water quality programs and is currently operating three wide-scale watershed projects to determine the effects of land uses on water quality. In these projects approximately 80 streams in each of three large geographical areas -- Illinois River Basin and adjacent areas, Little River Basin, and a large area draining south central Oklahoma, (hereafter referred to as the Muddy Boggy System) -- are being sampled to determine water quality. Sampling is conducted on a quarterly basis under normal flow conditions with the addition of two runoff event samples per year.

Based upon land uses, stream appearance, and water quality analysis, a number (approximately one-third) of the streams in each of these study areas were selected for biological assessment. Streams were selected from three categories: 1) those which appeared to be the least disturbed, 2) those streams which appeared to be the most disturbed and, 3) those streams which were in between the two extremes. This sampling design, where streams across a spectrum of water quality conditions are sampled, allows the measurement of the range of response to water quality conditions, identification of critical water quality thresholds, and establishment of reference conditions for the three study areas. Bioassessments, utilizing existing RBP protocols were conducted on these sites twice per year for benthic macroinvertebrates and once per year for fish. EPB samples were collected during the same period as other biological samples.

Chemical analysis of the six yearly water samples from the sites includes nitrate-nitrite, total kjeldahl nitrogen, total phosphorous, ortho-phosphate, total suspended solids, turbidity, alkalinity, chloride, sulfate, and hardness. Dissolved oxygen, specific conductance, pH, and temperature are measured in the field at the time of both chemical and biological sample collection. Chemical analyses were conducted at the USGS Geological Survey laboratory in Arvada, Colorado.

The streams selected for biological assessment primarily drain rural watersheds; however, some drain urban areas, and of these, a few receive discharge from municipal wastewater treatment plants. Stream watershed size varies but all are less than 15,000 acres in size. The small size of the watersheds facilitates the establishment of correlations between water quality and land uses by limiting the number of potential sources of pollution. Average stream width is approximately 10 feet and average depth is approximately one foot, although there is considerable variation

within each stream due to topography (pools, riffles). All of the study streams have a base flow of less than 10 cubic feet per second (cfs). See Figure 2 for the location of sites.

The goal of this research is to develop diatom-based stream assessment protocols based on information gathered during the OCC sampling program. Specific research objectives are:

1. Identification of the optimum season for sampling.

2. Identification of analytical procedures which optimize the speed at which assessments can be made.

3. Identification of the optimal sampling substrate.

4. Development of a series of assessment protocols.

5. Investigation into the relationship between chemical parameters and community response.

6. Investigation into ecoregional differences in diatom communities.

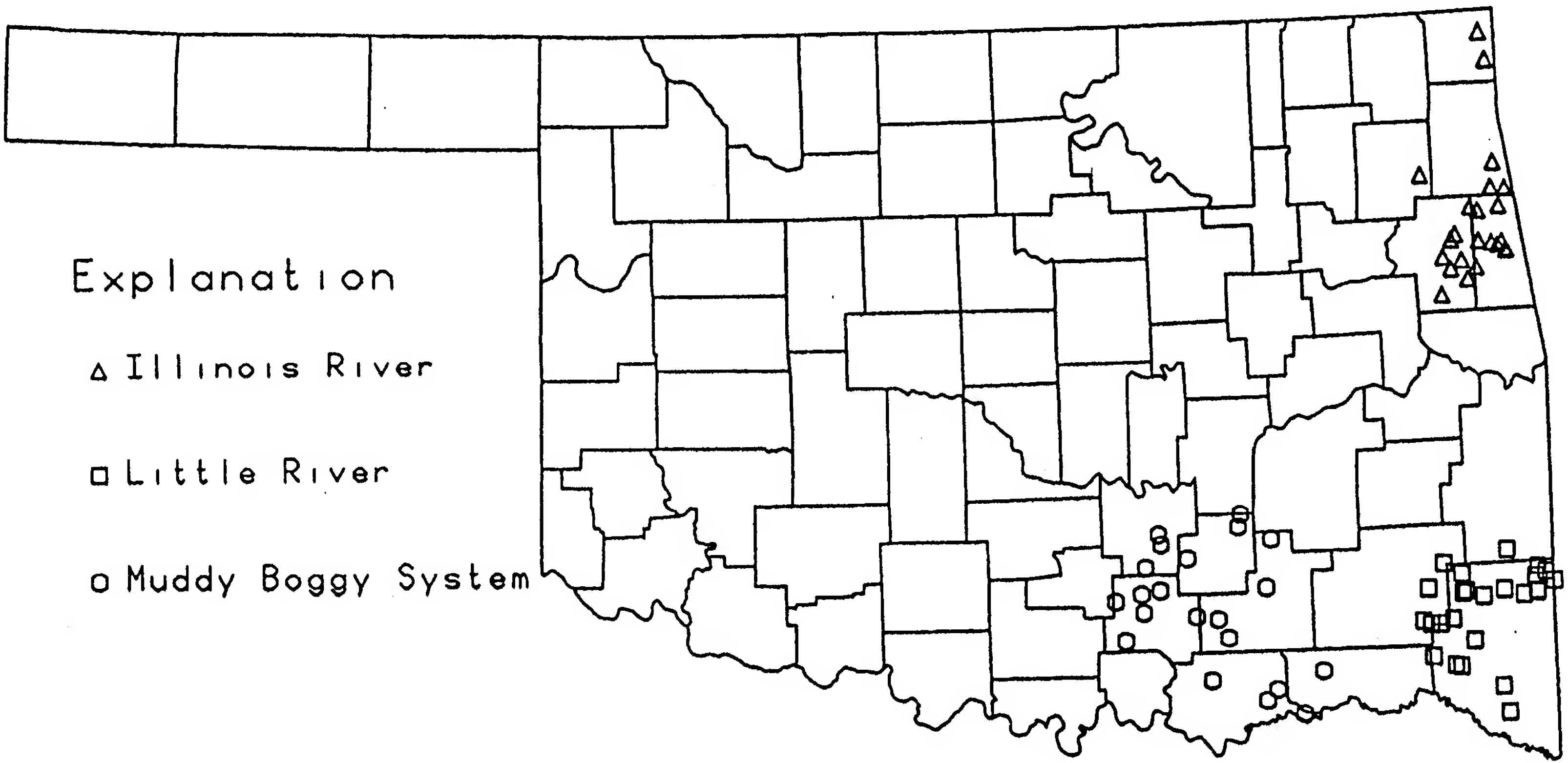

FIGURE 2. Biological Sampling Sites

CHAPTER 5

MATERIALS AND METHODS

Quantitative EPB samples were collected using artificial substrates which consisted of two glass rods (8 x 62 mm) suspended, approximately 3 inches apart, from a metal wire frame. Five samplers were placed at each site during each sampling period. Samplers were suspended by mono-filament fishing line from roots, limbs, fences, or other suitable materials. Great care was taken to insure that the samplers received equivalent sunlight and were in similar flow conditions; however, it was not possible to quantitatively control for these factors. The use of five samplers helped to insure that stream conditions were adequately measured; however, it should be noted that there were differences in the number of samplers recovered and that the multiple samples were not true replicates.

Samplers were always placed in areas of low stream velocity, but not in stagnant areas, as the substrates rely on drifting organisms for colonization, and re-supply of dissolved nutrients. The preferred location was a zone of low velocity immediately below a riffle. This insures adequate oxygenation of the water, and an abundant supply of drifting organisms due to the turbulent conditions immediately upstream.

A very similar substrate, glass tubes, was used by Meier et al. (1983) who found them to be an adequate tool for quantitative collection of diatoms with a lower coefficient of variance (CV) between tubes than with the more commonly used microscope slide or coverslips. Goldsborough et al. (1986) compared the CV reported for various periphyton community measurements on different substrates and found that CV when acrylic rods were used was lower than for most substrates. From this information it could be concluded that glass rods offer a convenient and representational substrate for colonization by lotic diatoms.

The artificial substrates remained in place for two weeks before retrieval. Different colonization periods have been suggested (Aloi, 1990; Cattaneo and Amireault, 1992); however, a two week period has been commonly used (Patrick et al., 1954; Aloi, 1990). Upon collection, the rods were placed in 6 oz. whirlpaks with the two rods from each sampler being placed in the same bag with a small amount of stream water (ca. 50 mL). Samples were placed on ice and returned to the laboratory for processing. It was unusual for all five samplers to be recovered intact due to a variety of factors including vandalism, storm events, animal damage, or predation. Rods which had obviously been grazed or otherwise predated upon were rejected as were those which had accumulated debris. The average return rate was 6.2 rods per site (62%) with

a minimum of zero and a maximum of 10.

Sample processing consisted of scraping the accumulated growth off of the rod with a section of longitudinally bi-sected tygon tubing (inner diameter - 8 mm). The scraped material was brought up to a consistent volume, generally between 100 and 150 mL. A portion of this volume was then filtered, ground, and analyzed for chlorophyll content according to Standard Methods for the Analysis of Water and Wastewater (1989). Final results are reported as micrograms of chlorophyll a per square centimeter.

The remaining portion was preserved with 2 mL of formalin prior to processing for microscopal analysis. All collected material from each site was composited and processed following the technique of Hasle and Fryxell (1970). An aliquot of cleaned sample was spread on a 25 x 25 mm glass coverslip and allowed to dry on a warming plate. The coverslips were then mounted on 1 x 3 inch glass microscope slides using Hyrax as a mounting medium. Two-hundred and fifty cells from each site were identified to the species level when possible; however, in some cases the orientation of the cell or uncertain taxonomy did not permit identification past genus. Cells were identified as encountered in lateral transects. In almost all cases, one slide contained several thousand diatoms; therefore, 250 cells were quickly encountered. Cells were observed at 1000x magnification utilizing an Olympus Model BH2 microscope, under phase contrast illumination. One hundred and seventy one samples were examined resulting in the identification of approximately 44,500 cells. A number of references were used in the identification of cells, and for particular species, confirmation from more than one source was very helpful. The following sources were used in these identifications:

The Diatoms of the United States (Vols. 1 & 2)
Die Susswasserflora Mitteleuropas (Vol. 10, 1930)
Diatoms of Alaska
Kieselalgen (Vols. 1 & 2)
Diatoms of Eastern Australia
Diatoms of New Zealand
Diatoms of Illinois
Die Diatomeen von Schweden und Finland
A Guide to the Common Diatoms at Water Pollution Surveillance System Stations
Susswasserflora von Mitteleuropa (Vols. 2/1 - 2/4)

The most complete and up-to-date of the above listed works is the latter one which is recommended for use by Round (1991). It is an update of the original series written in the early part of this century. A major disadvantage of these books is that they are written in German (with the exception of a helpful german/english term glossary); however, the four books contain over 9,000 figures (light and electron micrographs) which offset the disadvantages.

Although these books include many unusual and/or rare species which confuses species identification, they appear to make some important distinctions between species whose separation was previously less clear. The "Diatoms of Illinois" was found to be a helpful reference as it contained only those species which were commonly encountered. This focuses the book on the two to three hundred commonly occurring species while ignoring the thousands of rare ones.

Qualitative samples were collected by scraping rocks or other stream substrates. Only one sampling site, Pine Cr. in the Little River study area, did not contain cobbles and at this site tree roots were scraped. An important point made by Round (1991) is that is necessary to use 'a degree of subjectivity' in collecting from artificial substrates. Although it was seemingly not difficult to identify rocks which had been grazed or to distinguish between those which had been recently colonized or had been developing an epb community for a longer period, data analysis will prove otherwise. Substrates were scraped in the field or small cobbles were collected and returned to the laboratory where they were scraped. A stiff toothbrush functioned well for the purpose of scraping as described by Aloi (1990). Samples were preserved in formalin until processing according to the methods described above. Although natural substrates have been used for quantitative measurements (Aloi, 1990; Jacoby et al., 1991) (chlorophyll content, cell numbers) by scraping a measured area, this would not appear to provide an accurate quantitation of epb for several reasons:

1. The colonization period is unknown.

2. Natural substrates are very likely to have been grazed or otherwise predated upon.

3. The colonization period may allow for development of cyanobacteria or other non-diatom flora.

4. Influence of substrate textural variations.

5. Substrate types may vary between sites.

In addition, comparisons between sites are confounded by variations factors listed above. For reasons of comparing the qualitative nature of the diatom flora between sites, rock (or other substrate) scrapings may be adequate, especially if the primary indicator used is the dominance of indicator species or associations of dominant species (Round, 1991). One of the goals of this study was to determine if artificial substrates and naturally occurring ones provide similar information in rapid bioassessment protocols. The scraping of natural substrates has a major advantage in that only one site visit must be made, as opposed to the two visits necessary with artificial substrates.

CHAPTER 6

RESULTS

Several sets of data have been compared in the process of determining the adequacy and/or comparability of various sampling and analysis techniques. Comparisons are based upon parametric measurements of community structure such as diversity, dominance, richness, and evenness. The specific data sets which have been compared include:

A. Community analyses based upon different sample sizes.

B. Winter versus summer communities.

C. Communities from natural versus artificial substrates.

The existing higher level RBPs classify streams according to one of the following categories: non-impaired, slightly impaired, moderately impaired, or severely impaired, based upon their relationship to a eco-regionally specific reference (non-impaired) community. Since streams can be classified in only one of four ways, it can be inferred that small differences in community structure measurements are relatively un-important.

With stream classification divided into four classes (sometimes two or three) under existing RPBs, it would appear appropriate to base the assessment of the significance of the community measures discussed in this section on some fraction of either the measured or theoretical optimum value. For any given measurement of community structure the 'best' measure found within a particular data set would be considered to be 100%, or optimal, with some fraction of this value representing a significant difference. For example, if the Shannon Diversity Index is considered for all samples collected in the Illinois River Basin, the highest value found was 2.62, which could be considered as the optimally obtainable value (reference condition). The quarter value for this figure is 0.66 which could be considered as the cutoff value for the determination of significance. If this were the only measurement used in assessment then the following classifications could be used:

2.62 - 1.98 = non-impaired

1.97 - 1.32 = slightly impaired

1.31 - 0.66 = moderately impaired

0.65 - 0.00 = severely impaired

Changing any value by 0.66 units would automatically (100% of the time) move a value into the next quarter, and hence the next classification category. For the remainder of this discussion this value will be referred to as Qmax. The assumptions that are made with this approach is that Margalef and Mehinick Richness, Hills' numbers, Shannon Diversity Index, Shannon evenness, Sheldon evenness, Heip evenness, Hill evenness, and Modified Hill evenness all increase with an increase in the biological integrity of the community, while the Simpson Index decreases. A listing of the formulae used to calculate these indices is included in Appendix 4. A thorough discussion of the development and use of these indices is discussed in detail Ludwig and Reynolds (1988).

A. Sample Size Effects

The primary goal of this research was the development of stream assessment protocols which are relatively rapid yet produce accurate assessments of water quality conditions. The primary controlling factor determining the speed at which any biological assessment can be conducted is the number of organisms identified. For benthic macroinvertebrate protocols, a one-hundred organism sub-sample from the entire sample collected is identified. Fish protocols take into account the numbers of organisms which are captured; therefore, sample size varies from a few to several hundred individuals. The appropriate number of individuals to identify should be based on the level of information required and weighed against the time required for identification of larger numbers.

There was no attempt to find the "ideal" number of organisms in this study; however, from experience it was obvious that the identification of more that a few hundred organisms would remove the 'rapid' from the assessment protocols. Several researchers, most notably Patrick (1973), have suggested that large numbers of diatoms must be identified in order to accurately define the community and that the common practice of identifying 'only' 250 to 500 cells should not be relied upon until it can be mathematically proven that this number 'reflects the true structure of the community'. Patrick (1954) recommends that 8,000 cells from each sample be identified, although she demonstrates that very few taxa make up the majority of the community. This approach relies on a high level of taxonomic competence on the part of technicians where small inconsistencies can create major differences in results (Lange-Bertalot, 1979).

While it may be difficult to argue against this recommendation in terms of the importance of identifying every species of diatom present in a sample, it may not significantly increase the amount of water quality information as opposed to a smaller sample size. Over-counting is viewed by Round (1991) as 'the excessive searching for and counting of vast numbers (>400) of the few abundant species

confused by multitudes of casual species'. Van Dam (1982) states that 'the structure of diatom assemblages is most often characterized by the presence of one of a few, very abundant species and a rather limited number of rare species'. Castenholtz (1960) found very similar community structure measures when 300 organisms as opposed to 1000 organisms were identified.

There are many practical reasons for looking at a smaller number of organisms:

1. Very few analysts (including the author) would be willing to spend days on one sample looking for that one last species and it is even recognized by Patrick (1973) that identifying large numbers is laborious.

2. Identification of thousands of individuals from each sample greatly increases the cost of any program and biological sampling programs have been historically underfunded.

3. It is doubtful that the presence of only a few individuals of several species would significantly affect the results of numerical manipulations of the data such as species diversity, richness, or percent dominance by the major species.

4. The identification of this many organisms removes the "rapid" from bioassessment protocols.

5. Fish and benthic macroinvertebrate communities are characterized by the most abundant species present, and this has been shown to be true of the diatom community as well.

6. In most cases, only a relatively small number of species make-up a large percentage of the population (Round, 1991; Descy, 1979). This is the group of interest, not those whose presence is relatively minor.

7. Assessment protocols could not practically be based on the presence of rare species as they would be found too seldom to make assessments feasible.

In this study, approximately 250 organisms were identified from each sample. On thirty-six of these samples, an indication was made when the first 100 organisms had been identified. Sample parameters such as diversity, evenness, species number, and richness were then calculated for individual samples when 100 organisms versus 250 organisms were identified.

The results of these comparisons are listed in Table 1. As can be seen there was very little difference in the stated measures of community structure. The average number of new species found after the first 100 organisms had been identified was 6.1 with a maximum of 14 and a minimum of 1 additional species. The average quantity of these additional species was 1.8 organisms with a maximum of 5.0 and a minimum of 1.0 organism.

Table 1. Differences in community measures between 100 and 250 organism cell counts (Qmax = 1/4 of maximum value measured; n=36).

Measure	Qmax	Mean ¦D¦ (250-100)	Mean D (250-100)
Margalef R -	1.63	0.62	0.56
Mehinick R -	0.62	0.25	0.19
Hills N0 -	9.00	6.08	6.08
Hills N1 -	5.62	0.73	1.14
Hills N2 -	9.03	0.07	0.76
Simpson Index -	0.23	0.03	0.01
Shannon Index -	0.78	0.13	0.09
Shannon E -	0.22	0.05	-0.04
Sheldon E -	0.16	0.09	0.09
Heip E -	0.16	0.33	0.33
Hill E -	0.20	0.04	-0.04
Mod. Hill E -	0.20	0.05	-0.05

Analysis of this data set reveals some of the problems associated with statistical inference. For Shannon's Diversity Index, the mean difference between a 100 and 250 organism sample was 0.09 units, with a mean absolute difference of 0.13 units. According to both the Wilcoxon ranked signs test and the matched pair-t, the two (100 vs 250) sets of data are significantly different ($p<0.01$); however, this does not pass the test of ecological significance as this represents only a very small change in community structure

(Qmax = 0.78). This holds true for the other parameters listed in the above table, as well. One interesting observation is that the smaller sample size generated higher evenness values on over 80% of the samples tested. This was likely due to the fact that the average number of new species was close to 1.

Another important measure of community structure is the dominance by one or a few species. This can be measured through the Simpson Index which ranges in value from 0 to 1.0. From the above table, it can be seen that the average differences between different sample sizes was 0.01 with an absolute mean difference of 0.03. As mentioned before this very small and ecologically insignificant difference is indicated to be statistically significant by the Wilcoxon Ranked sign and matched pair t-tests; however, it does not approach the Qmax value of 0.23. This indicates that the relative dominance of dominant species was not different between the two sample sets. This is confirmed by the raw data as the dominant organism(s), after 100 organisms had been identified, was invariably still dominant even after an additional 150 organisms were identified.

Conclusions:

From these data it is apparent that the measures of community structure discussed here (with the exception of the Heip Evenness Index) were not significantly different, as defined by Qmax, in an ecologically important sense when 100 organisms versus 250 organisms were identified. This would appear to pass Patrick's own test of proving mathematically that the smaller number 'reflects the true structure of the community'. It would be interesting to extend this test from very small numbers into the thousands to determine the level of change in community structure.

It can be concluded that no significant additional information is obtained from looking at 250 versus 100 organisms and that a 100 organism sub-sample is adequate to demonstrate population characteristics. In terms of time needed for analysis this represents a great savings.

B. Summer Versus Winter Collections

Seasonal differences in diatom communities are important in the selection of sampling dates. Ideally the best season for study is when populations are at their highest diversity; however, an argument can be made that when fewer taxa are present, water quality effects might be more pronounced. The goal of this aspect of protocol development was to determine whether summer and winter diatom populations differed in taxonomic structure, production, and hence usefulness in assessment protocols. Differences in production between seasons will be discussed in a subsequent chapter. There are several advantages and disadvantages to

sampling in both summer and winter. The primary advantages of winter sampling are the relative absence of shading by riparian vegetation, fewer high flow events, less competition from non-diatom algae and riparian vegetation, and a decreased potential for disturbance by humans. Disadvantages of winter include a lower sun angle, shorter days, colder water, and less intense agricultural land uses.

The data from forty-two sites where summer and winter samples were collected and examined for differences in community structure are listed in Table 2.

Table 2. Differences in community measures between summer and winter collections for all study areas (Differences (D) represent summer minus winter values; Qmax = 1/4 of maximum value measured; SD = standard deviation; n=42)

Measure	Min. D	Max. D	Mean D	SD D	Qmax
Margalef R	-3.70	6.19	0.48	1.95	1.82
Mehinick R	-1.22	2.07	0.17	0.65	0.63
Hills N0	-21	35	2.7	11.0	10.5
Hills N1	-8.25	28.79	1.46	7.14	6.00
Hills N2	-7.79	24.71	0.69	5.33	4.89
Simpson Index	-0.63	0.74	<0.01	0.27	0.22
Shannon Index	-2.07	2.29	0.11	0.89	0.77
Shannon E	-0.58	0.53	0.02	0.23	0.21
Sheldon E	-0.29	0.36	0.02	0.18	0.16
Heip E	-0.32	0.41	0.02	0.20	0.15
Hill E	-0.35	0.35	-0.02	0.15	0.21
Mod. Hill E	-0.43	0.34	-0.01	0.18	0.20

The mean differences between summer and winter values are small and do not approach Qmax values; however, individual differences did exceed Qmax. The standard deviation of D is large relative to the mean which indicates that there was considerable variance in the data. Taken as a whole these data show that on average the structure of diatom communities, as measured by these indices, is not significantly different, based on Qmax, between summer and winter, although in some specific cases the differences are very pronounced. The data are inconclusive concerning the season in which the best information can be obtained; however, the fact that most measures were higher in summer would tend to indicate summer samples tend to be richer, more diverse, and slightly more even.

The sample set represents data from each of the three study areas. When the data concerning summer versus winter values are looked at by individual study area it can be seen that there are marked differences (Table 3).

Table 3. Differences in community measures between summer and winter collections by study area; (differences (D) represent mean summer minus mean winter values; Qmax = 1/4 of maximum value measured)

	Muddy Boggy System		Little River		Illinois River	
	Mean		Mean		Mean	
	D	Qmax	D	Qmax	D	Qmax
Margalef R	2.26	1.75	0.08	1.42	-0.41	1.04
Mehinick R	0.76	0.60	0.02	0.49	-0.12	0.36
Hills N0	12.70	10.10	0.54	8.25	-2.40	6.13
Hills N1	5.59	5.99	1.70	3.73	-1.29	3.06
Hills N2	2.57	4.82	1.39	2.68	-0.90	2.34
Simpson Index	-0.04	0.21	-0.12	0.15	0.08	0.18
Shannon Index	0.48	0.77	0.32	0.67	-0.23	0.62
Shannon E	0.05	0.21	0.11	0.20	-0.02	0.20
Sheldon E	0.03	0.15	0.09	0.13	-0.02	0.14
Heip E	0.04	0.14	0.09	0.12	-0.03	0.13
Hill E	-0.08	0.21	<0.01	0.21	<0.01	0.20
Mod. Hill E	-0.04	0.19	0.08	0.20	-0.03	0.19

Differences in summer versus winter values were highest in samples from the Muddy Boggy system. Mean values were higher than Qmax values for both richness indices and Hills N0; however, all other values were small in comparison to Qmax. Most notable in these data is that summer samples contained an average of 12.7 more taxa than winter samples. Evenness values were very similar across seasons as indicated by the small mean differences. Samples taken from the Little River also tended to have higher values for these measurements in summer versus winter although differences were not as pronounced as for the Muddy Boggy system, and did not approach Qmax values. Again evenness values were very similar across seasons.

The most interesting values from the above table concern the samples from the Illinois River. The measurements from this area were almost always higher in the winter than summer, although the differences did not approach Qmax values for any measurement.

It is not readily apparent as to why these areas differ so markedly in respect to relative changes in community structure across seasonal extremes. From this data it can be concluded that the best season in which to collect diatoms cannot be predicted a priori. Seasonal differences can be extreme at given sites and in no case should samples collected during different seasons be compared for the purpose of making water quality assessments.

Conclusions:

From the data collected, it would appear that communities are likely to be more diverse, rich, and even during summer months; therefore, given a lack of evidence concerning the optimum season for a particular study area, summer months should be chosen for collection of diatom samples. Statistical analysis of community distribution using canonical correspondence analysis supports this conclusion which will be discussed in detail in Chapter 9. Summer sampling is also preferred for fish and benthic macroinvertebrates which alleviates some logistical concerns as all three communities can be sampled during the same period. If the timing of an environmental event is known, such as seasonal pesticide or fertilizer applications, then sampling should be scheduled in order to measure the effects of the particular event.

C. Artificial Versus Natural Substrates

One of the research goals of this project was to determine the relative efficiency of artificial versus natural substrates in collecting diatoms for stream assessments. Most members of the EPB community are not selective in their habitat requirements, especially the diatoms, and it has been shown that the material used for the artificial substrates has little consequence in affecting the species composition of the EPB community (Cattaneo and Amireault, 1992). A review of the literature found that the only group which demonstrated substrate selectivity was the cyanobacteria; however, this appeared to be at least partially due to the length of time that the substrates were allowed to be colonized. Aloi (1990), Round (1991), and Cattaneo and Amireault (1992) recommend naturally occurring substrates as a means of eliminating any substrate bias and suggest that there are significant differences between the communities which develop on artificial versus natural substrates. Patrick (1979) found that the distribution of the most common taxa were very similar between natural substrates and glass slides which was quantified when Patrick (1973) showed that 95% of the species present on natural

substrates were also found on glass slides. Hudon et al. (1987) found quantitative differences between the communities which developed on different substrates but no differences in species diversity. This study attributed quantitative differences to variation in surface texture with rougher surfaces accumulating more periphyton.

In terms of logistics, natural substrates offer a great advantage over artificial substrates in that only one trip to a site is required, and the frequently occurring loss of natural substrates to natural events and vandalism is eliminated.

In order to address this question, natural substrates were sampled at the time that artificial substrates were recovered in both summer and winter. Details concerning sample collection can be found in the materials and methods section. Differences in these communities were compared using the same measures of community structure as were used in other comparisons. In addition, differences in taxonomic structure were investigated.

The data comparing natural versus artificial substrates are listed in Table 4. For all data, with the exception of the Simpson Index, differences were derived by subtracting natural substrate from artificial substrate value; therefore, a negative number indicates that the natural substrate yielded a 'better' value. For the Simpson Index, the inverse should be assumed. From these data it can be seen that the diatom community structure, as illustrated by these measures, was not very different when all data was considered; however, for individual samples the differences were often large. The standard deviation was large relative to the mean differences in all cases which indicates that there was a considerable variation among responses at different sites.

Table 4. Differences in community measures between natural and artificial substrates. Listed values represent artificial substrate minus natural substrate measures. (SD = standard deviation; #-(%) and #+(%) represent the number of individual differences which were neg. and pos., respectively).

Parameter	Mean D	Min. D	Max. D	SD	# -(%)	# +(%)	Season
Marg. R	-0.56	-3.25	1.82	1.28	66.7	33.3	W
	-0.11	-2.66	2.96	1.60	57.9	42.1	S
Meh. R	-0.18	-1.02	0.64	0.44	66.7	33.3	W
	0.05	-0.88	0.96	0.53	57.9	42.1	S
Hills N0	-3.3	-19	10	7.2	66.7	33.2	W
	-0.5	-15	17	9.0	57.9	42.1	S
Hills N1	-2.47	-14.4	8.51	5.80	70.8	25.9	W
	-0.51	15.8	24.50	9.51	52.6	47.4	S
Hills N2	-1.26	-8.54	6.19	3.93	54.2	45.8	W
	-0.16	-12.40	23.90	8.05	47.4	52.6	S
Simpson Index	-0.11	-0.48	0.28	0.22	62.5	37.6	W
	-0.01	0.38	0.30	0.19	47.4	52.6	S
Shannon Index	-0.41	-1.95	1.07	0.82	66.7	33.3	W
	0.01	-1.22	1.38	0.76	52.6	47.4	S
Shannon E	-0.11	-0.48	0.28	0.22	62.5	37.5	W
	0.01	-0.31	0.35	0.18	42.1	57.9	S
Sheldon E	-0.08	-0.38	0.24	0.18	62.5	37.5	W
	0.01	-0.36	0.39	0.18	36.8	63.2	S
Heip E	0.09	-0.42	0.25	0.19	62.5	37.5	W
	0.01	-0.37	0.40	0.20	47.4	57.9	S
Hill E	0.01	-0.32	0.24	0.13	37.5	62.5	W
	<0.01	-0.28	0.38	0.19	36.8	63.2	S
Modified Hill E	-0.06	-0.40	0.34	0.19	54.2	45.8	W
	-0.01	-0.34	0.44	0.21	47.4	57.9	S

Natural substrates produced higher (better) values for all richness and diversity measures, although the mean differences are not large. In terms of the actual number of samples where natural substrates produced higher values it can be seen that this occurred approximately two-thirds of the time in the winter and slightly over half the time in the summer. The data in Table 4 also points out that the different substrates consistently produced values which were more similar in the summer than in the winter, although it will be demonstrated that taxonomic differences were greater in the summer. It appears likely that increased grazing was at least partially responsible for these differences which will be demonstrated when taxonomic differences are examined. Evenness values were also relatively similar between the two substrates; however, there is no consistent differences between seasons for the five measures listed. Three evenness indices indicate that the two substrates were more similar in summer, while two indicate the opposite. Despite this inconsistency, the mean differences are relatively small, although some individual values were large and would exceed Qmax values if this approach were taken.

There were important differences between artificial and natural substrates in terms of taxonomic composition, the magnitude of which varied between the seasons. In summary, the data showed that:

1) Natural substrates were often dominated by species which have been reported to be resistant to grazing (low electivity, i.e. Achnanthes spp.) due to their small size and prostrate growth habit (Gregory, 1983; Steinman et al., 1992)

2) Artificial substrates in the winter were dominated by species with rapid immigration rates (Synedra spp., Diatoma vulgare, Melosira varians, and Meridion circulare (Stevenson and Peterson, 1989; Oemke and Burton, 1986; Stevenson et al., 1991).

Table 5 contains data concerning the percentage of populations that were made up of Achnanthes spp. on artificial versus natural substrates during summer and winter collections.

Table 5. Percent of Population Composed of Achnanthes spp. in Winter and Summer on Artificial and Natural Substrates.

a. Summer

	Substrate		
	Natural	Artificial	Difference
Min.	1.1	0.0	0.8
Max.	86.7	24.6	84.6
Mean	31.4	6.5	25.7
SD	32.3	6.5	31.0

b. Winter

	Substrate		
	Natural	Artificial	Difference
Min.	0.0	0.0	0.0
Max.	68.9	45.8	59.3
Mean	28.1	10.1	19.9
SD	24.5	15.0	16.9

These data demonstrate that Achnanthes spp. were more abundant on natural substrates which is probably attributable to differences in grazing pressure (electivity). In only three of eighteen samples in the summer and two of twenty-two in the winter were more Achnanthes spp. found on artificial substrates, all of which occurred when percentages were low on both. The high standard deviation and the range of differences suggests that this difference is highly variable. It can be concluded from this data that the probability of collecting a sample from a natural substrate which has been grazed is high and that the use of artificial substrates would lessen the potential for this source of sampling error.

The fact that natural substrates show signs of grazing activity in the summer should not be surprising. Insect, molluscan, and fish grazers were abundant at many of these sites and signs of grazing on cobbles and bed rock material were obvious. Although the measures of community structure are not very dissimilar during summer between artificial and natural substrates, species composition was very different. The probability of collecting a sample from a natural substrate which was not significantly altered by grazing activity seems small; therefore, the conclusion that can be drawn is that assessment using natural communities may yield

biased results as species composition may be determined more by the selectivity of grazers than water quality. Grazing on artificial substrates can occur; however, there was little visual evidence that this had occurred while it was obvious on the natural substrates. From this I would conclude that artificial substrates would consistently yield a community structure which was less biased by grazing activity.

It would be assumed that grazing pressure is less during the winter due the relative inactivity of grazing organisms; however, these data suggest that grazing effects are approximately equal. It has been demonstrated that a significant portion of drifting organisms are released due to grazer activity; therefore, the pool of potential immigrants would be expected to be smaller during winter. The most efficient immigrants are species which have a large length to width ratio and these organisms have also been reported to have high electivity indices (Steinman et al., 1992; Stevenson et al., 1991; Stevenson and Peterson, 1989; Oemke and Burton, 1986). Their morphology and electivity may account for their preponderance on artificial substrates during the winter and the relative lack of other species. Of the twenty-two samples collected during the winter where both substrates were examined, species with high immigration rates were found as shown in Table 6.

Table 6. Proportion of Population Composed of Species with High Immigration Rates (% of total). (Difference = Artificial Minus Natural Substrate).

	Natural Substrate	Artificial Substrate	Difference
Min.	0.0	0.4	0.4
Max.	54.1	92.1	56.7
Mean	12.4	35.0	22.5
SD	16.2	29.4	22.1

There was a considerable difference among samples as evidenced by the high standard deviation; however, on average, a larger percentage of species with high immigration rates was found on artificial rather than natural substrates. In addition the mean difference between the two substrates was almost a quarter (22.5%) of the total number of organisms counted, and high immigrant rate species were more abundant on natural substrates only three out of twenty-two times.

On samples taken during the winter, the dominant species was the same on both artificial and natural substrates 50% (11 of 22) of the time while during the summer, only 4 out of 19 (21%) samples had the same dominant species on both substrates. Interestingly, of the four samples which had the same dominant species during the summer, it was the same species, Cocconeis placentula, in all cases. This species has been reported as have a relatively low electivity value, which could explain these similarities.

Conclusions:

Although several conclusions have been drawn from this particular set of data, the primary conclusion is that artificial substrates are less likely to suffer the effects of predation. In light of the fact that there are considerable differences in electivity among species, it is likely that the community structure will be altered by grazing and may be a better indicator of grazing pressure than water quality. In some cases communities were very similar between natural and artificial substrates; however, it is not known if this was due to the lack of grazers or the chance collection of a non-grazed area. If the magnitude of the difference between artificial and natural substrates is due entirely to grazing pressure, this may offer some indication of the health of the grazing community, although this might be difficult to quantify.

In the event that sampling by means of artificial substrates is not possible, the data should be closely analyzed to determine if grazing effects are present and any such data should be discarded. The data indicate that natural substrates have a more complex community structure during winter than during summer, perhaps due to a decrease in grazer activity; therefore, natural substrates may be adequate tools during winter. Every effort should be made to avoid grazed areas during sample collection. Heavily grazed areas are obvious and although they were avoided in these collections, the success of this effort was demonstrably inadequate. This may have in part been due to the size of the area of natural substrate sampled.

A frequent observation in streams is that molluscan grazers prefer more stable substrates such as large boulders or bedrock areas. In order to circumvent these areas, at least two small rocks were chosen for scraping, with an average diameter between three and five centimeters. It may be that the areas of small rocks provided a better refuge for non-molluscan grazers whose evidence of activity is less visibly noticeable. Descy (1979) in developing his assessment protocol sampled approximately the same area (25 cm^2); however, sampling was in rivers where filter feeders would be expected to be the most abundant invertebrate as opposed to smaller streams where grazers dominate (Minshall et al., 1992,

Vannotte et al., 1980). A researcher knowledgeable in differences between grazers and filter feeders (differences which are obvious on a gross anatomical scale and easily distinguishable) might be able to discern if natural substrates are appropriate based on a cursory examination of the benthic macroinvertebrate fauna.

Since different streams or stream reaches will be compared under assessment protocols it would be beneficial if confounding factors were kept to a minimum and the use of artificial substrates would lessen the effects of substrate bias. Taken together this information indicates that artificial substrates should be used when at all possible; however, for lower level protocols, qualitative sampling of natural substrates may be adequate.

CHAPTER 7

DEVELOPMENT OF ASSESSMENT PROTOCOLS

Rapid bioassessment protocols classify streams as non-impaired, slightly impaired, moderately impaired, or severely impaired, based upon their relationship to eco-regionally specific reference (non-impaired) conditions. Lower level protocols may eliminate the slightly impaired category or both the middle categories. Diatom-based protocols will use similar classes in order to facilitate the integration of all three groups into an overall stream bioassessment.

The classification of a stream into one of the four categories is usually based upon a comparison to reference sites. Reference sites (conditions) have been established in each of the study areas for their use in these comparisons; however, the lowest level protocol may not use reference conditions as a basis for comparison.

There are several reasons for developing more than one protocol for a biological community. Higher level protocols are intended to provide a more thorough or more accurate assessment when this is required or when more information is available. Lower level protocols may be used when financial, equipment, time resources or the taxonomic abilities of the investigators are limited, or on those occasions when more accurate information is not required. Three protocols will be developed for the diatom community utilizing the community measures (metrics) discussed in detail in the following sections. The discussion of these metrics is focused on the highest level protocol (#III) which classifies streams into one of four categories. In these cases the metric is divided into quarter values. When a similar metric is used for Protocol II, which classifies streams into one of three categories, the metric will be divided into third values. This distinction will be made clearer when each protocol is established.

For some of the metrics discussed, a degree of impairment is included to demonstrate the concept of assessing streams based upon the distribution of metric scores. Stream classification utilizing individual protocols is based upon the results of several metrics; therefore, impairment classifications are included only for the purpose of illustration.

A. Indicator Species

Two factors have been discussed in regard to indicator species. One is the observation that certain species, when found in abundance, are indicative of water quality conditions. The second is that there may be differences in indicator species on some geographical level. The biotic indices developed by Descy (1979) and Lange-Bertalot (1979) were both developed from rivers and it may be that these will need some refinement if they are to be applied to small streams. Many species of diatoms have been shown to have a cosmopolitan distribution and an initial review of the literature has demonstrated that particular species are almost invariably listed as indicators of poor water quality. There is much less emphasis in the literature on those species which indicate un-polluted water, although there is some agreement on the value of particular genera. There would appear to be a sufficient data base on which to draw conclusions from both extremes.

The list of species which were found to be dominant during this study is included in Appendix 1. Species which are indicated as tolerant are given a value of three, those which are rated as intolerant are designated as one and those which fall between these two extremes are given a value of two. The development of this list was complicated by the fact that the same species have been listed as having different indicating value by different authors. In most cases it has been possible to sort out these discrepancies based upon a preponderance of evidence. In those cases where no information was available, a species was given a default value of two. Dominance in this case has been defined as the five most numerous species present or that number which make up 90% of the population, if this number is less than five. In addition, for a species to be considered as dominant, it must have contributed >10% to the total number of cells identified. Fifty-five species met these requirements.

It is probably not justified by either time or ecological merit to consider the indicator value of every species found at a site when calculating the Diatomic Index. The species which dominate the sample should be considered; however, this will vary between samples. Additionally, the more species that are considered the greater the likelihood of encountering a species for which little or no indicator information is available. The number of species which made up the majority of population were examined in forty samples with the results listed in Table 7.

Table 7. Number of species composing 75% and 90% of population (n=42; IR = Illinois River; MB = Muddy Boggy System; LR = Little River)

	Number of species						
	Min.	Max.	Mean	Mean % of Total	IR Mean	MB Mean	LR Mean
90%	1	30	9.4	39.7	6	11	11
75%	1	17	5.1	22.2	-	-	-

On average, 9.4 species made up 90% of the population although there was considerable variation among samples. As will be seen in the following section, on average, one species accounts for approximately 50% of the population. In order to account for 75% of the population, one would, on average, have to consider 5.1 species. Neither of these values represents a significant burden in terms of time involved; however, a large number of relatively dominant species could be overlooked if diversity and richness were high. Because of this it is probably better to consider the mean percent of total when choosing the appropriate number of species to include when calculating a Diatom Index.

The number of species which made up 90% of the population averaged 39.7% of the total number of species while for 75% of the population this value was 22.2%. In the worst case scenario in this study, this would mean that the indicator value would be considered for 30 species, while it would average 6 in Illinois River, 11 in the Muddy Boggy System, and 11 in the Little River (Table 7). These averages would appear to be low enough to warrant gathering information on the species which make up 90% of the population. It should be noted that some minimum number should be established below which a species does not warrant consideration. In these calculations no species which accounted for less than 1% (2.5 individuals) of the population was included. This would appear appropriate; however, if only one hundred organisms are counted a higher percent value should be established to exclude consideration of those species which were found only once. I would suggest that a species must be found at least twice in a sample for its indicator value to be considered. The use of a Diatom Index takes into account the relative abundance of species; therefore, any species which occur infrequently will be given a smaller weight.

The Diatomic Index developed by Descy (1979) assigns an indicator value (five levels) to individual species as well as a measure of the 'ecological amplitude' (three levels) of individual species. This results in the potential for fifteen different levels of classification which would not appear to be justified based on the amount of supporting chemical data. Lange-Bertalot (1979) divides diatoms into three tolerance levels which appear to be equivalent to Descy's three ecological valence categories; therefore, the division of tolerance into three classes has historical precedence. The formula used for calculating this index from the data gathered in this study is:

$$\text{Diatom Index} = \sum \text{Iv} \times \text{Rf}$$

where: Iv = indicator value of each species
Rf = relative frequency (%) of each species

B. Species dominance

One of the simplest measures of community structure is the percent of the population made up of the most dominant species. The larger this number is, the more uneven or dominated the community would be. A non-reference comparison value could be based upon either the percent of the best sample found during this study or on a theoretical basis. Theoretically if 100 organisms were identified the best value possible would be 1%. It is extremely unlikely that 100 organisms would be encountered with out any redundancy, but again taking the Qmax approach, one could base classification on a theoretically perfect sample as follows:

% dominance	Classification
0 - 25%	un-impaired
26 - 50%	slightly impaired
51 - 75%	moderately impaired
76 - 100%	severely impaired

A more realistic approach is to look at the best sample within each study area and to base the value on this practical maximum. For the three study areas the data is shown in Table 8.

Table 8. Percent of population composed of dominant taxon.

	Max.%	Min.%	Mean%	SD
Illinois River	97.2	34.7	48.1	17.8
Little Rive	92.1	16.1	36.0	18.7
Muddy Boggy	96.9	8.2	48.6	23.6
All samples	97.2	8.2	44.6	20.8

From these data it can be seen that in the extreme the dominant taxon accounted for nearly 100% of the organisms identified and that the percentage was similar for the three study areas. With such a small difference between the observed maxima and 100%, deviation from a classification based upon 100% dominance is probably not warranted and should be appropriate when reference conditions are not available.

A different way that these data could be used would be to base classification on the best conditions found which would be more in keeping with previously established community metrics. For the Illinois River area, as a minimum, the dominant taxon accounted for 34.7% of the sample and, conversely, 65.3% of this sample was composed of other taxa. In relationship to this optimum or reference condition streams could be classified as:

	% Dominance		
Classification	**Muddy Boggy System**	**Little R.**	**Illinois R.**
un-impaired	8.2 - 31.0	16.1 - 37.0	34.7 - 51.0
slightly imp.	31.1 - 54.0	37.1 - 58.0	51.1 - 67.3
moderately imp.	54.1 - 77.0	58.1 - 78.9	67.4 - 83.6
severely imp.	77.1 - 100	79.0 - 100	83.7 - 100

Dominance by a single species was as common in the summer as the winter; however, the average dominance was slightly higher (48.2%) in the winter than in the summer (41.3%). The mean dominance was very similar between the Illinois River and Muddy Boggy System (48.1%, 48.6%) but somewhat different for the Little River System (36.0%). The reasons for this difference are unclear but point out that there may be significant ecoregional differences.

C. Percent Dominance x Indicator Value

In order to stress the importance of the dominant taxon as a measure of stream impairment, its presence should be give extra weight. This can be accomplished by developing a separate metric which multiplies the percent dominance by the indicator value of that species. This metric would not be used in Protocols II and III which employ a similar concept in the Diatom Index, but would be used in Protocol I to provide some information concerning the "indicator value" of the dominant taxon. The discussion of dominant species has been based on the premise that a high dominance by a single species is an indication of poor water quality. While this is almost invariably true, there are situations (<u>very</u> low nutrients) where the dominant species might be an indicator of high quality water. The incorporation of an indicator value would ameliorate the effect of relying totally on the percent dominance.

D. Measures of Community Structure

Measures of community structure were employed in comparing different sampling protocols; however, their limitations have already been discussed as they relate to their use as indicators of community integrity. Two measures of community analysis have been suggested: 1) Indicator Species and 2) Percent Dominance. These concepts are relatively simple in comparison to diversity, richness, or evenness indices, unfortunately, they are incomplete in their ability to assess the community as a whole. Diversity and evenness are important measures of community structure despite the limitations of various indices; therefore, one or more of the available indices should be included in an assessment protocol.

The use of community structure indices is well discussed by Ludwig and Reynolds (1988), Washington (1984), and Boyle et al. (1990) who point out their strengths and weaknesses. Ludwig and Reynolds (1988) recommend either Hills N1 or N2 as measures of community diversity and the Modified Hill Evenness as a measure of evenness. Hills N1 is a measure of the abundant species in a sample (based on the Shannon diversity index) while N2 measures very abundant species (based on Simpsons index). According to Ludwig and Reynolds (1988), N2 is preferred by most ecologists; therefore, this index will be used as the measure of community diversity.

The number of species (Hills N0) is used in other bioassessment protocols as a measure of community structure; however, this measurement disregards relative abundances, and in this study a large number of species were only found once per sample. Due to the fact that diatoms can drift at the mercy of the current for considerable distances and are commonly brought in on foreign material (i.e. birds) unlike fish or macroinvertebrates, it would appear that the presence of a single organism should be discounted or given very little weight; therefore, this measure of community will be used in only the lowest level protocol.

Ludwig and Reynolds recommend the Hill Evenness Indices over others as they represent the ratio of very abundant to abundant species using the Shannon and Simpson indices. The Modified Hill Evenness Index approaches zero as a single species becomes dominant; therefore, it is suggested that this is better measure of evenness.

I. Number of species (taxa)

The number of species (taxa) found at a site offers a degree of information concerning the structure of the community; however, it is limited in that it does not take into account the relative proportions of those taxa. Despite this limitation, it would appear to have usefulness in lower level protocols. It is obviously not possible to calculate a theoretical maximum value; however, a standard can be based on the values which were found in this study. The classification in regard to this metric for Protocol I will be discussed in the section dealing with that protocol. For Protocol II, which relies on comparisons to reference conditions and only uses three levels of classification, the following divisions can be made:

	# of species		
Classification	**Muddy Boggy System**	**Little R.**	**Illinois R.**
un-impaired	51 - 34	41 - 28	25 - 17
moderately imp.	33 - 16	27 - 13	16 - 8
severely imp.	$\leq$16	$\leq$13	$\leq$8

II. Hills N2

The significance of most metric values in each stream can be looked at in terms of their relation to either maximum (or minimum) observed or theoretical values. In the case of N2 it is difficult to determine theoretical maxima and there may be a considerable range across ecoregional boundaries. As demonstrated by the differences in maximum values found in the three study areas: 28.06, 10.89, 16.89 this measure should not be used unless reference conditions are present. When reference conditions are available, the value for a particular stream should be judged as before using the Qmax concept. For the three study areas this would translate to:

Classification	**Muddy Boggy System**	**Little R.**	**Illinois R.**
un-impaired	28.06-21.05	16.89-12.68	10.89-8.18
slightly imp.	21.04-14.03	12.67- 8.45	8.18-5.46
moderately imp.	14.02- 7.01	8.44- 4.23	5.45-2.73
severely imp.	<7.00	<4.22	<2.72

III. Modified Hill Evenness

As with Hills N2, an ideal value is difficult to predict; however, there may be a theoretical minimum and maximum dependent upon the sample size. For the three study areas the minima and maxima were: 0.36/0.81, 0.29/0.89, 0.30/0.82. These values are very similar; therefore, it may be reasonable to assume that the highest practically obtainable value approaches 0.90, although theoretically these values can exceed 1.00. The minimum values occurred when one species accounted approached 100% of the population. A theoretical community composed of 249 members of one species and one member of a second species was calculated to have a modified Hill index of 0.30. Given this observation is difficult to imagine that the value would often be below 0.30; therefore, this value will be used as the theoretical minimum. Despite the consistency of these data and the relative predictability of minima and maxima, it is suggested that this measure only be used when reference conditions are available. For the study areas the classification would work out as follows:

Classification	Muddy Boggy System	Little R.	Illinois R.
un-impaired	0.89 - 0.74	0.82 - 0.69	0.81 - 0.68
slightly imp.	0.73 - 0.59	0.68 - 0.56	0.67 - 0.55
moderately imp.	0.58 - 0.45	0.55 - 0.43	0.54 - 0.43
severely imp.	0.44 - 0.30	0.42 - 0.30	0.42 - 0.30

IV. Cairns Sequential Comparison Index (SCI)

The SCI was developed by Cairns et al (1968) and Cairns and Dickson (1971) as a simplified measure of community diversity where:

SCI = number of runs/number of taxa assessed

This method is appropriate for the lowest level protocol in that it does not rely on extensive taxonomic abilities of the analyst. The analyst compares each organism encountered with the previous one and assesses whether they are the same or different taxa. A run begins when successive taxa are judged to be the same and ends when a different taxon is encountered. The analyst need not identify either organism as a specific taxon and it is relatively easy to separate most species (and all genera) when they are both in the same field of view.

This test also offers an advantage in that a theoretical maximum exists (1.0) when each organism encountered is different and a minimum of (1/N) when each organism encountered is the same. For screening protocols which classify streams as either impaired/un-impaired, the calculated SCI could be categorized as:

Classification	SCI
un-impaired	1/n - 0.5
impaired	0.51 - 1.0

If the analyst has a higher level of taxonomic ability, this index can be improved by including a measure of the number of taxa encountered. This diversity index (DI) is calculated as:

DI = SCI x number of taxa

According to Cairns and Dickson (1971), polluted streams will have a DI of less than 8.0 while healthy streams will have a DI of greater than 12.0. If we remove the middle values the results could be classified as:

Classification	**DI**
un-impaired	>10.0
impaired	$\leq$10.0

E. Biomass Measures

Another important measure of diatom community response to water quality conditions is the mass of cells present after the incubation period. There are several methods available for measuring the abundance of organisms present including cell counts, productivity, and measures of standing crop. Different methods are reviewed by Clark et al. (1979).

The simplest of these is the cell count. Given that a known area of substrate has been collected, it is easy to calculate the number of organisms per unit area through cell counts. The disadvantage of this method stems from the fact that diatoms differ widely in cell volume. Many species in the genus Achnanthes are less than five by three micrometers in valve surface area while some species in the genus Synedra are several hundred micrometers long. As a result, when counts from different sites are compared, the actual number of cells present may be a poor indication in the amount of biomass present. Cell volume measurements have been used as means to address this problem; however, these measures are inaccurate and very time consuming.

A second type of commonly use method involves measurements of community productivity. This can be accomplished through various techniques with the most common being production/respiration (P/R) measurements or uptake of radioactively labelled carbon or phosphorous. P/R measurements are time-consuming, involve a large experimental set-up if a number of sites have been sampled, and must be conducted relatively soon after sample collection. Labelling studies are usually conducted in the lab; however, their primary disadvantage is expense.

The most common methods used for measuring standing crop are biomass as weight or chlorophyll a content. The measurement of biomass as weight is relatively simple and inexpensive but has a disadvantage in that it measures the weight of all material present. This can be corrected by the use of ash-free weight measurement; however, this will also include bacteria, fungi, or any other biological organisms present.

The advantages of chlorophyll a as a measure of biomass include: preservability of samples through freezing, relatively low cost, and a restriction of the measurement to photosynthetic organisms. The disadvantage of chlorophyll a measurements is that they do not separate out that chlorophyll a which is produced by algae other than diatoms, although as previously mentioned, diatoms make up the majority of the organisms on artificial substrates.

The use of chlorophyll a as a community metric is based on the assumption that increased chlorophyll levels indicate increased levels of nutrients. It has already been mentioned that an increase of nutrients above non-detectable levels can increase species diversity and stream production must be maintained at some minimum level in order to have a diverse fish and macroinvertebrate community. The data will show that the effects of changing the concentration of nutrients (nitrogen and phosphorous) is significantly correlated with chlorophyll standing crop in some cases.

Chlorophyll a values in three study areas were distributed as shown in Table 9.

Table 9. Distribution of chlorophyll values in all study areas.

	Mean Site values (ug/cm^2)				
	Min.	Max.	Mean	1st Quart.	4th Quart.
Illinois R.	0.55	7.05	2.68	<1.70	>2.95
Little R.	0.22	4.24	1.53	<0.62	>2.00
Muddy Boggy	0.14	10.15	2.05	<0.52	>2.92
			Mean	0.95	2.76

Although mean values for the study areas may not be valid as indicators of differences in ecoregional conditions, they do indicate that there are differences in production between the study areas. There was a considerable range among chlorophyll a concentrations within ecoregions although it is difficult to predict if the higher values represent ecoregional maxima. Maximum chlorophyll a values at individual sites were evenly distributed between summer (52.6%) and winter (47.7%) samples.

In the absence of data concerning the optimum or detrimental levels of chlorophyll a, an data-based approach will be taken to define classification values. This aspect of protocol development is subject to the highest degree of subjective interpretation; however, decisions will be based on the data collected. Classification values developed in this section may be too restrictive as in the case where the measured range of chlorophyll a values was limited (Little River) or too liberal but it should be re-stated that assessment protocol criteria are not static and can be adjusted as new information becomes available. As previously mentioned a chlorophyll a value below a certain level could be considered as detrimental, and in some collections levels below detection limits were encountered, although this was rare. Another factor which should be considered is that low chlorophyll a values may result from the presence of phytotoxins and not from low nutrient levels. The potential for toxins to be present should be carefully considered during data assessment especially in cases where sites fall below industrial discharges or are in the drainage of urban areas.

The data show that the mean cut-off value for the first quartile ranged from 1.70 to 0.52 ug/cm^2 with a mean of 0.95. Of the samples analyzed, mean chlorophyll values less than 1.00 ug/cm^2 ranged from 16.0% to 41.7% of the samples between study areas with an average of 31.4% for all samples. From this data it can be concluded that: 1) a chlorophyll a concentration of less than 1.00 ug/cm^2 is a reasonably obtainable goal and, 2) a value of 1.0 ug/cm^2 is approximately equal to the first quartile of the data distribution. Although it is not possible to determine if this value represents a desirably low level, it will be chosen as the cut-off for determining a lack of impairment based on the data distribution.

Mean values represent a range of seasonal values and a considerable range among artificial samplers at some sites for a single sampling date; therefore, given this range of data, it would appear to be appropriate to round off average values to the nearest whole unit when setting category thresholds. A value of 1.0 ug/cm^2 is lower than that found as the first quartile cutoff in the Illinois River study area; however, since nutrient levels are higher in this area than the other two the data distribution is skewed towards higher values. It may be found in other areas that this value represents an unobtainably low level of chlorophyll or falls in the upper end

of the distribution spectrum and in these cases, an ecoregionally specific value should be chosen.

The selection of the other classification values should be based upon the fourth and intermediate quartile values from each study area. Because of the variation in values, rounding to the nearest whole unit would again appear to be in order. The fourth quartile values are listed in the above table and all three lie very close to or on a whole unit. Taken together chlorophyll a values would be used in classification as follows:

Chlorophyll a (ug/cm^2)

Classification	Muddy Boggy System	Little R.	Illinois R.
un-impaired	≤1.00	≤1.00	≤1.00
slightly imp.	>1.00-2.00	>1.00-1.50	>1.00-2.00
moderately imp.	>2.00-3.00	>1.50-2.00	>2.00-3.00
severely imp.	≥3.00	≥2.00	≥3.00

CHAPTER 8

ASSESSMENT PROTOCOLS

Bioassessment protocols are meant to provide information concerning water quality by determining the biological integrity of the analyzed communities. Some words of caution should be put forward for those contemplating the use of the protocols set forth in this document as well as others which have already been developed. Precautionary language has already been developed by USEPA and states that protocols have been "...designed to provide basic aquatic life data for planning and management purposes such as screening, site ranking, and trend monitoring. All of the protocols utilize fundamental assessment techniques to generate basic information on ambient physical, chemical, and biological conditions. Level of assessment and level of effort vary with successive protocols, and choice of a given protocol should depend on the specific objective of the monitoring activity and available resources. Although none of the protocols are meant to provide the rigor of fully comprehensive studies, each is designed to supply pertinent, cost-effective information when applied in the appropriate context" (Plafkin et al., 1989).

A. Protocol I

Protocol I assessment is intended to be used as a screening tool to identify areas of impairment/non-impairment without comparison to reference conditions. This protocol should be very rapid and can be used to identify areas where additional investigation is warranted. Although this protocol does not require identification to the species level, it is based on the ability of the analyst to separate taxa. This ability will obviously vary between individuals; however, the quality of all assessment protocols are subject to limitations imposed by the ability of the taxonomist; therefore, this should not introduce any errors which have not been previously encountered. Even at this level of bioassessment it is inappropriate for individuals without training in taxonomy to conduct the analyses. Metric a in this protocol is based on the ability of the analyst to identify the dominant taxon to species level. If this ability is lacking, this metric can be discarded.

Methods:

Qualitative sampling only

Identification to lowest practical level

No comparison to reference conditions (site)

Metrics:

a. Percent dominance by most numerous species

b. Percent dominance x indicator value

c. Number of taxa present

d. Cairns sequential comparison index (SCI) or diversity index based on SCI

a. Percent Dominance by most numerous taxon

Given that the maximum percent dominance within each basin was near 100% and that the mean percent dominance was approximately 50% it would appear to be appropriate to classify these values as follows:

% dominance		Score
0 - 50%	=	2
51 - 100%	=	1

b. Percent Dominance x Indicator Value

Indicator values range from one to three, with one indicating the best water quality conditions. If the percent dominance is multiplied by this score, the theoretical minima and maxima are:

1% (0.01) x 1 = 0.01

100% (1.00) x 3 = 3.00

Values can be scored as:

% dominance x value		Score
0.01 - 1.50	=	2
1.51 - 3.00	=	1

c. Number of taxa present

The number of taxa (species in this case) found during this study ranged between three and thirty-one when 100 organisms were counted with a mean of 17.5 and a median of 17.0. For this metric it would appear to be inappropriate to establish the mid- point of the range as the cutoff value as 17 taxa can represent a diverse community. A more conservative approach is to take the first quartile value, 12, as the cutoff value which results in the following scoring scheme:

# taxa		**Score**
>12	=	2
≤12	=	1

d. Cairns Sequential Comparison Index (SCI)

The use of the SCI index has already been discussed in detail and will be scored as follows:

SCI		**Score**
1/n - 0.50	=	2
0.51 - 1.00	=	1

Tabulation of Metrics

When all of the community metrics in protocol I are tabulated the potential values range from 4.0 to 8.0. Stream quality will be based on the following division of these values:

Score		**Stream Classification**
8.0 - 6.0	=	un-impaired
<6.0 - 4.0	=	impaired

B. Protocol II

Protocol II will be based upon comparison to reference conditions and will classify streams as un-impaired, moderately impaired, or severely impaired according to the following metrics:

Methods:

Quantitative sampling

Identification to species level

Comparison to reference conditions (site)

Metrics:

a. Percent dominance by most numerous species

b. Number of species present

c. Cairns Diversity Index (based on SCI)

d. Diatom Index

a. <u>Percent Dominance by most numerous species</u>

The score for this metric is based upon the minimum percent dominance (MD) as indicated by the reference conditions. In the examples for the study areas examined in this project this was accomplished by establishing quarter percent values. Since this protocol will classify streams according to one of three categories, it is more appropriate to divide the values into third values (TV) where:

$$(100\% - MD)/3 = \text{Third values} = TV$$

Range of Values		Score
<MD - (MD + TV)	=	3
(MD + TV) - (MD + 2TV)	=	2
(MD + 2QV) - 100% dominance	=	1

b. <u>Number of species present</u>

This metric is calculated by dividing the maximum number of species found (reference condition) by three and is then scored as:

Range of Values		Score
Max. # species - 2/3 Max.	=	3
2/3 Max. - 1/3 Max.	=	2
<1/3 Max.	=	1

c. <u>Cairns Diversity Index (CDI)</u>

The use of the index has already been discussed in detail and will be scored as follows:

CDI		Score
Max. DI - 2/3 Max.	=	3
2/3 Max. - 1/3 Max.	=	2
<1/3 Max.	=	1

d. <u>Diatom Index</u>

The Diatomic Index is calculated as previously described and is compared to the reference condition. The theoretical minimum and maximum for this index under the conditions where those organisms which make-up ≥90% of the population are used is 0.01 and 3.00; however, the actual reference values should be used where:

(3.00 - Min. value)/3 = third value (TV)

The stream is then scored according to the following scheme where:

Range of Values		Score
0.01 - TV	=	3
TV - 2TV	=	2
>2TV	=	1

Tabulation of Metrics

When all community metrics are tabulated the streams will be classified according to their numerical relationship to the maximum obtainable value. The theoretical minimum and maximum values are 4.0 and 12.0; however, classification should be based upon the measured maximum score (reference condition) where:

% of reference		Stream Classification
100 - 66%	=	un-impaired
65 - 33%	=	moderately impaired
≤33%	=	severely impaired

C. Protocol III

Protocol III incorporates additional measures of community structure plus a measure of community production. Given the additional information streams are classified into one of four categories: un-impaired, slightly impaired, moderately impaired, and severely impaired based upon comparisons to reference conditions.

Methods:

Quantitative sampling

Identification to species level

Comparison to reference conditions (site)

Metrics:

a. Percent dominance by most numerous species

b. Percent dominance x indicator value

c. Hills N2 diversity index

d. Modified Hill Evenness

e. Chlorophyll measurement

f. Diatom Index

a. <u>Percent Dominance by most numerous species</u>

The score for this metric is based upon the minimum percent dominance (MD) as indicated by the reference conditions. In the examples for the study areas examined in this project this was accomplished by establishing quarter percent values:

(100% - MD)/4 = Quarter values = QV

Streams are then scored according to the following values:

Range of Values		Score
<MD - (MD + QV)	=	4
(MD + TV) - (MD + 2QV)	=	3
(MD + 2QV) - (MD + 3QV)	=	2
>(MD + 3QV)	=	1

b. <u>Percent Dominance x Indicator Value</u>

When all samples are considered, this value ranged from 0.08 to 2.92 which approaches the theoretical minima and maxima. The score of this metric in relation to reference conditions is calculated where the reference condition equals the minimum value according to the following calculation:

(100 - MD) x indicator value = reference value (RV)

and

(100-RV)/4 = quarter values (QV)

This metric is then scored as:

Range of Values		**Score**
<RV - (RV + QV)	=	4
(RV + QV) - (RV + 2QV)	=	3
(RV + 2TV) - (RV + 3QV)	=	2
>(RV + 3QV)	=	1

c. Diatom Index

The Diatomic Index is calculated as previously described and is compared to the reference condition. The theoretical minimum and maximum theoretical values for this index under the conditions where those organisms which make-up 90% of the population are used is 0.01 and 3.00; however, the actual reference values should be used where:

(3.00 - Min. value)/4 = quarter value (QV)

The stream is then scored according to the following scheme where:

Range of Values		**Score**
0.01 - QV	=	4
>QV - 2QV	=	3
>2QV - 3QV	=	2
>3QV	=	1

d. Hills N2

Stream scores are based on their relationship to reference conditions where QV is equal to a quarter of the maximum value measured. Individual streams are then scored as:

Range of Values		Score
Max. - 3/4 Max.	=	4
3/4 Max. - 1/2 Max.	=	3
1/2 Max. - 1/4 Max.	=	2
<1/4 Max.	=	1

e. Modified Hill Evenness Index

As discussed before, the lowest measurable value for this index under the conditions of these analyses approaches 0.30; therefore, this number should be used as the minimum value unless a smaller number is measured. The theoretical maximum can exceed 1.00, although this would appear to be unlikely with a practical obtainable maximum of approximately 0.90. If a number of streams have been sampled in an area and an estimation of the obtainable maximum is available, this number should be used. If this number is unavailable, a value of 1.0 should be used. Stream scores are based on the quarter values of the maximum range (MR) or 1.00 to 0.30 where:

Range of Values		Score
MR - 3/4 MR	=	4
3/4 MR - 1/2 MR	=	3
1/2 MR - 1/4 MR	=	2
<1/4 MR	=	1

f. Chlorophyll a

The use of chlorophyll as a measure of community production was discussed in some detail where it was shown that a concentration of 1.00 ug/cm^2 appeared to be both an achievable minimum and an approximate value for the first quartile. In the absence of sufficient evidence which demonstrates that this value is unobtainably low for an ecoregion, it should be used as the cutoff value for the highest score. The succeeding scores were based upon quartile values for the data in each of the study areas. Due to the fact that it is unlikely that such a large number of streams would be studied in all cases and that quartile values may be impossible to determine, some absolute number must be established when quartile values are not available. Taken as a whole, and by individual study areas, it appears that a value of 3.0 ug/cm^2 is a reasonable fourth quartile value; therefore, it should be used in the absence of information which indicates otherwise. If adequate information exists, the maximum range (MR) of values should be divided by four with each quarter representing a different score as demonstrated below:

Range of Values	Chlorophyll a ug/cm^2)		Score
MR - 3/4 MR	<1.00	=	4
3/4 MR - 1/2 MR	>1.00 - 2.00	=	3
1/2 MR - 1/4 MR	>2.00 - 3.00	=	2
<1/4 MR	>3.00	=	1

Tabulation of Metrics

Final stream scores are based upon the sum of individual metrics with their classification based upon their numerical relationship to reference conditions. The theoretical minimum and maximum values are 6.0 and 24.0; however, classification should be based upon the measured maximum score (reference condition) where:

% of reference		Stream Classification
100 - 75%	=	**un-impaired**
74 - 50%	=	**slightly impaired**
49 - 25%	=	**moderately impaired**
<25%	=	**severely impaired**

CHAPTER 9

CORRELATION OF BIOLOGICAL AND CHEMICAL DATA

The goal of this research is the development of stream assessment protocols so that impaired streams can be identified using characteristics of the diatom community. Chemical analyses were made at each site and the results bear some discussion as they can identify the source of impairment in some cases. This information will also help validate some of the conclusions regarding the results of impairment assessments on individual streams. This information can also be used in the development and refinement of indicator species information. For example, Cocconeis placentula is listed by both Lange-Bertalot (1979) and Descy (1979) as being a relatively intolerant organism; however, in this study, this organism was almost invariably found to be dominant when stream nutrient levels were the highest. Similar information can be developed for other previously classified species as well as those which have not been given an indicator values.

A. Linear Relationships

Chemical and community structure data was explored to determine if significant linear relationships existed between these parameters. Linear relationships were determined between various data sets using the Spearman Rank Correlation test with a p value of <0.05 designated as the level of significance. The data were examined as a whole, and by study areas and seasons. Table 10 lists the associations of the data sets under various combinations.

Table 10. Linear relationships between nutrients and chlorophyll production where:

TP = average total phosphorous concentration (mg/L)
TN = average total nitrogen concentration (mg/L)
Chl = average chlorophyll concentration (ug/cm^2)
S/NS(p) = statistically sig./non-sig. (p value)

	Relationships		
Data set	TP/TN	TN/Chl	TP/Chl
I. All Data	S(<0.0001)	S(<0.0001)	S(0.0020)
Little R.	S(0.0001)	NS	S(0.0165)
Illinois R.	S(0.0077)	S(0.0312)	S(0.0176)
Muddy Boggy	S(0.0005)	NS	NS
II. Summer data	S(<0.0001)	S(0.0013)	S(0.0250)
Little R.	S(0.0005)	NS	S(0.0073)
Illinois R.	S(0.0064)	NS	NS
Muddy Boggy	S(0.0025)	NS	NS
III. Winter data	S(<0.0001)	S(<0.0001)	S(<0.0001)
Little R.	NS	NS	S(0.0160)
Illinois R.	S(0.0168)	S(0.0394)	S(0.0016)
Muddy Boggy	S(0.0037)	NS	NS

When taken as a whole the data show that significant relationships exist between TN/TP, TN/Chl, and TP/Chl. It is not surprising that phosphorous and nitrogen are related in a linear sense as it is difficult to imagine a scenario where the addition of one would occur without the addition of the other. The significance of the relationships between nitrogen and phosphorous concentrations and chlorophyll standing crop is encouraging as the measure of chlorophyll is intended as a biological surrogate for nutrient measurement; however, it should be pointed out that other nutrients may play a significant role in determining chlorophyll levels. This relationship holds during summer and winter although it appears to be stronger during winter which may be attributable to increased competition for nutrients during summer.

The nutrient data which was collected in this study tends to be clustered at both ends of the spectrum, with relatively few points in mid-range. Although the inclusion of mid-range data points would not be expected to change the significance of the relationship, it could have a significant effect on the slope of the line of best fit; therefore, the existing data should be used cautiously, if at all, in predicting quantitative response of chlorophyll production.

The results concerning the relationship between nutrient levels and chlorophyll standing crop is less conclusive when looked at in terms of individual basins. If all data are considered for individual study areas it can be seen that only in the Illinois River area was the relationship significant for both TN and TP, while TP was significantly related to chlorophyll in the Little River area. No relationships were significant in the Muddy Boggy study area nor the data as a whole or during either season, which indicates that other factors are responsible for determining production levels and that neither phosphorous nor nitrogen are limiting on a basin-wide basis.

Relationships are less significant when the data are examined in terms of seasonal differences. During the summer collection period, the only significant association found was between phosphorous and chlorophyll in the Little River area. The absence of significant relationships in the other two areas indicates that other factors are more responsible for determining chlorophyll standing crop than nitrogen and phosphorous during the summer. A significant relationship between phosphorous and chlorophyll was found in the Little River and in the Illinois River study areas during winter with a significant relationship existing between nitrogen and chlorophyll in the Illinois River. This indicates that phosphorous plays an important role in determining chlorophyll levels in these basins.

In conclusion it can be stated that chlorophyll standing crop is related to nitrogen and phosphorous levels during some seasons and in some areas; however, other factors appear to exerting a controlling influence in some cases. If one excludes light, current, temperature or other physical factors from the consideration, then it can be inferred that other chemical species are playing an important role in stream productivity. It can also be inferred from this that nitrogen and phosphorous are not, as commonly assumed, the only limiting factors for algal growth.

B. Multivariate Analysis

Multivariate analyses were accomplished using the canonical correspondence analysis (CCA) techniques developed by Ter Braak (1986, 1987, 1988). These techniques allow for the simultaneous examination of the association between species, site, and environmental factors. The data generated in this study will be examined as a whole by season, by study area, and in the context of inter-area differences. Data were log transformed prior to analysis in order to down-weight the importance of species with high abundance.

I. Data by Season

CCA analysis of the entire data set by season offers further evidence that summer collections provide superior data. As seen in Figure 3, study area sites are more tightly clustered by study area during summer than during winter (corresponding site names are included in Appendix 2). This indicates that eco-regional differences are more pronounced during warmer months; therefore, this would be the preferred period for sampling. This supports the species data which indicates that summer populations provide superior information; therefore, additional CCA analyses will be restricted to data collected during summer.

For the summer data, there are three sites, L40, i17, and i18, which lie well outside of their respective clusters and are separate from the data distribution as a whole. These three sites are located below sewage treatment plants and should be expected to be significantly different. The clustering of these three sites indicates that the magnitude of sewage discharge effects are similar across ecoregional boundaries. These sites are atypical of the other study sites within the study two areas and have extreme weights in CCA outputs. Because of these weights, they tend to skew the remainder of the data set; therefore, they have been removed for data analysis of individual study areas. There are additional sites which are not well separated into their respective study-area clusters which may indicate that they lie in ecoregional transition zones or are experiencing some unusual environmental pressure(s). It can also be observed that Illinois River sites are more closely clustered which indicates that conditions were more uniform across this study area than the other two.

Since the three study areas have been demonstrated to be separate entities in terms of the variables measured, analysis of species-environment relationships will be conducted by study area.

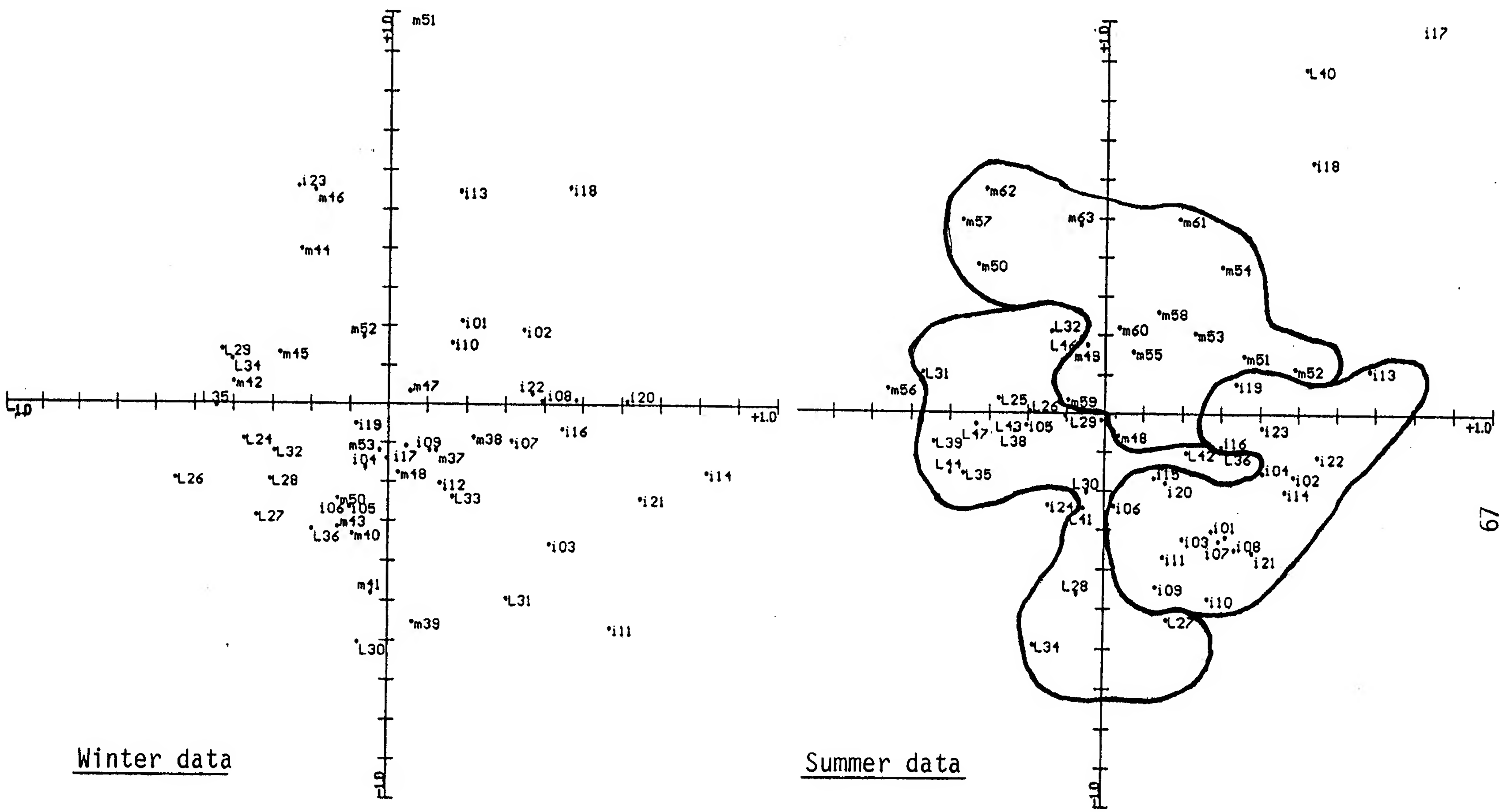

Figure 3. Scatter plot of site distributions. m# = muddy boggy site #, i# = Illinois River site #, 1# = Little River site #

II. Data by Study Area

a. Illinois River

CCA plots of Illinois River data concerning species/nutrient relationships are seen in figure 4. Species abbreviations for all areas are included in Appendix 3. Several species demonstrate an association with increasing nutrient levels: Cymbella mexicana, Gomphonema augur, Nitzschia frustulum, Gomphonema minutum, Gomphonema parvulum, Gomphonema subtile, and Cymbella affinis. Others appear to be associated with lower nutrient levels: Achnanthes lanceolata, Achnanthes marginulata, Cymbella minuta, Fragilaria fasciculata, Gomphonema pumilum, Meridion circulare, and Synedra ulna. Only one species, Navicula cryptocephala, was closely associated with increasing chlorophyll levels while three species were strongly associated with low chlorophyll values: Eunotia bilunaris, Epithemia adnata, and Rhopalodia gibba. This may indicate that some species are arranged along the chlorophyll gradient according to competitive ability. Navicula cryptocephala is a notorious indicator of poor water quality and, as evidenced by this data, is a good competitor as it occurs when chlorophyll values are high. Epithemia adnata and Rhopalodia gibba appear to be poor competitors based on this data. Since these two species have been demonstrated to have nitrogen fixing capabilities, it appears that competitive abilities were lost in exchange for the ability to exist in low nutrient/low competitive environments.

Four species were associated with increasing diversity levels (Figure 5): Meridion circulare, Fragilaria fasciculata, Achnanthes linearis and Eunotia bilunaris while others: Gomphonema utae, Cocconeis placentula, Nitzschia frustulum, Gomphonema minutum, and Gomphonema subtile were associated with low diversity values. The latter species were also associated with increasing nutrient levels which supports the supposition that increasing nutrient levels results in a decrease in diversity. This is also seen in the fact that Meridion circulare and Fragilaria fasciculata were associated with low nutrient conditions and high diversity values.

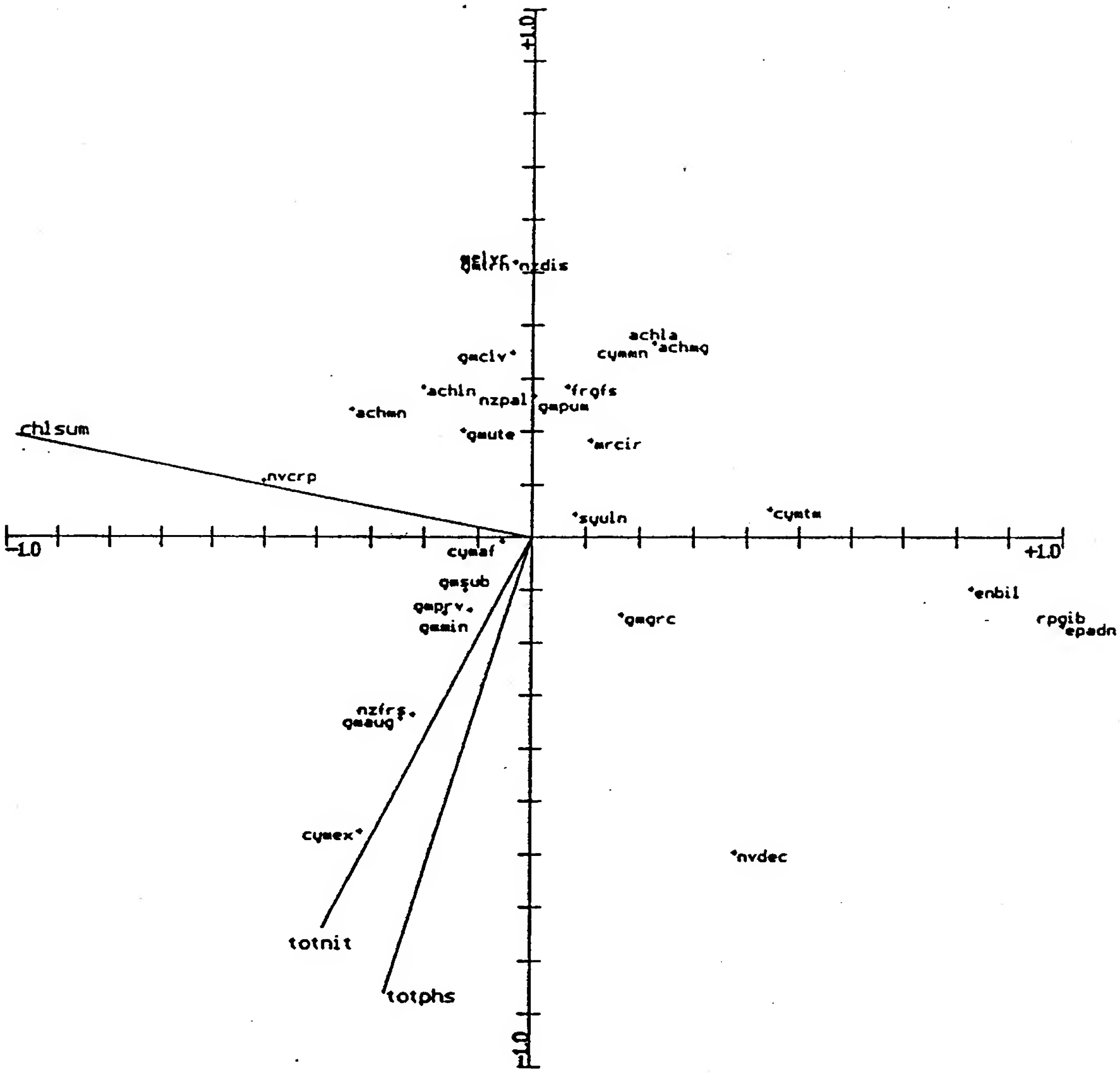

Figure 4. CCA biplot. Illinois River. Species/nutrients/chlorophyll
totphs = total phosphorous, totnit = total nitrogen, chlsum = chlorophyll

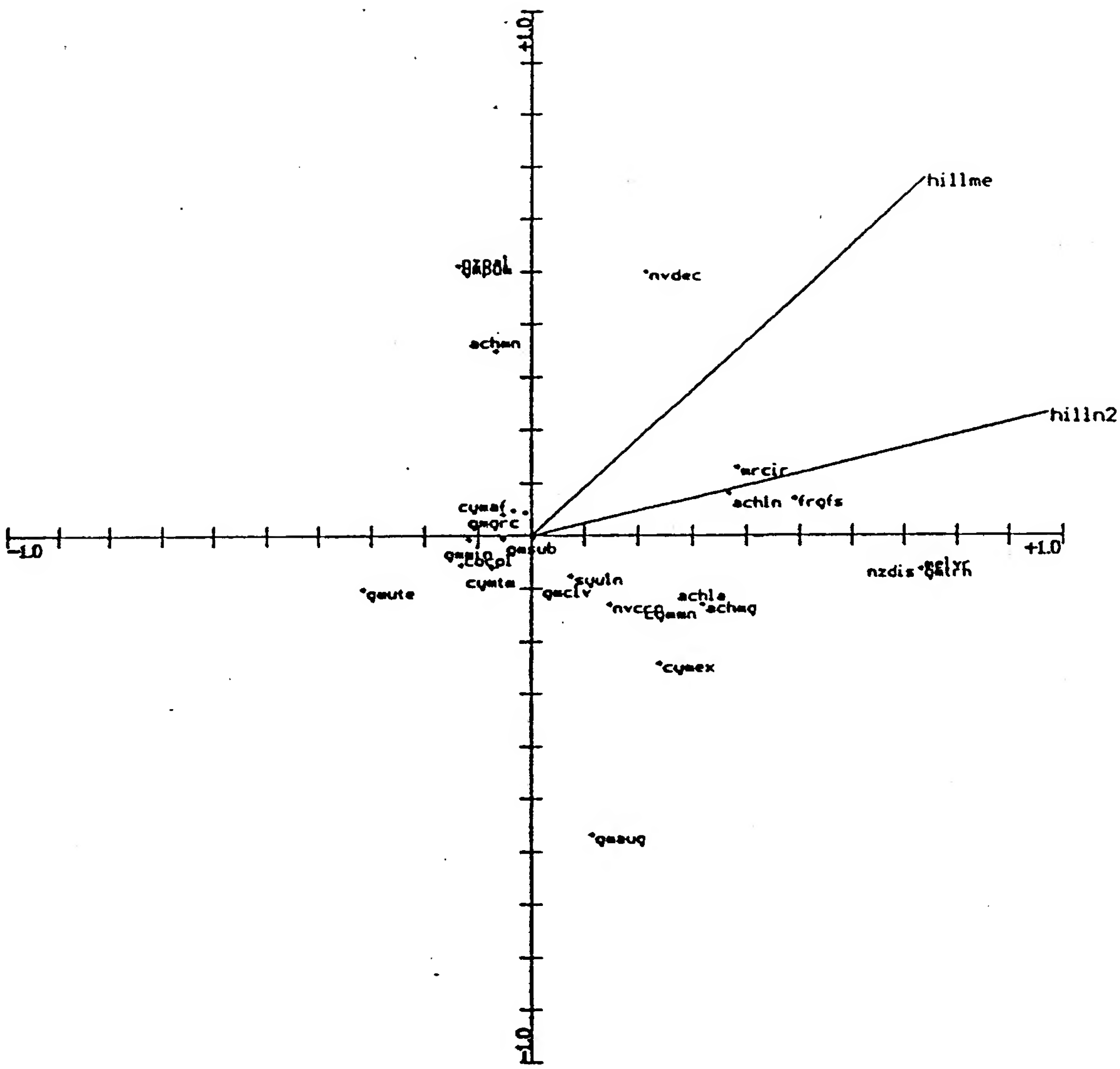

Figure 5. CCA biplot. Illinois River. Species/community measures. hillme = Hills modified eveness, hilln2 = Hills N2 diversity index

A summary of the CCA output is contained in Table 11. From the first section of this table it can be inferred that the first axis is primarily a chlorophyll gradient while the second and third axes are primarily nutrient gradients. Species/environment correlations were similar for the three axes. For the measures of community diversity, it can be seen in the second section of the table the first axis was primarily associated with changes in Hills N2 diversity index while the second axis was associated with the Modified Hill Evenness Index.

Table 11. Summary of CCA output - Illinois River

	Axis 1	Axis 2	Axis 3
Eigenvalues	0.345	0.215	0.133
Species/Environment corr.	0.873	0.737	0.823
Variable correlations:			
Total Nitrogen	-0.337	-0.543	0.457
Total Phosphorous	-0.236	-0.633	-0.357
Chlorophyll a	-0.857	0.143	-0.024

	Axis 1	Axis 2
Eigenvalues	0.323	0.137
Species/Environment corr.	0.845	0.662
Variable - correlations:		
Hills N2	0.817	-0.168
Modified Hill Evenness	0.597	-0.468

b. Little River

Similar gradients were seen in the Little River study area as shown in Figures 6 and 7. Several species were associated with increasing nutrient levels, two quite strongly, Eunotia arcus and Cymbella aspera (Figure 6). Several species were associated with low nutrient values; however, none of these associations were particularly strong. Stronger associations are seen along the chlorophyll gradient with eight species being strongly associated with increasing chlorophyll levels and several others associated with low chlorophyll values.

The associations between species and environmental gradients as diversity and evenness indices is less pronounced although some species are clearly associated with high and low diversity numbers and low evenness values (Figure 7).

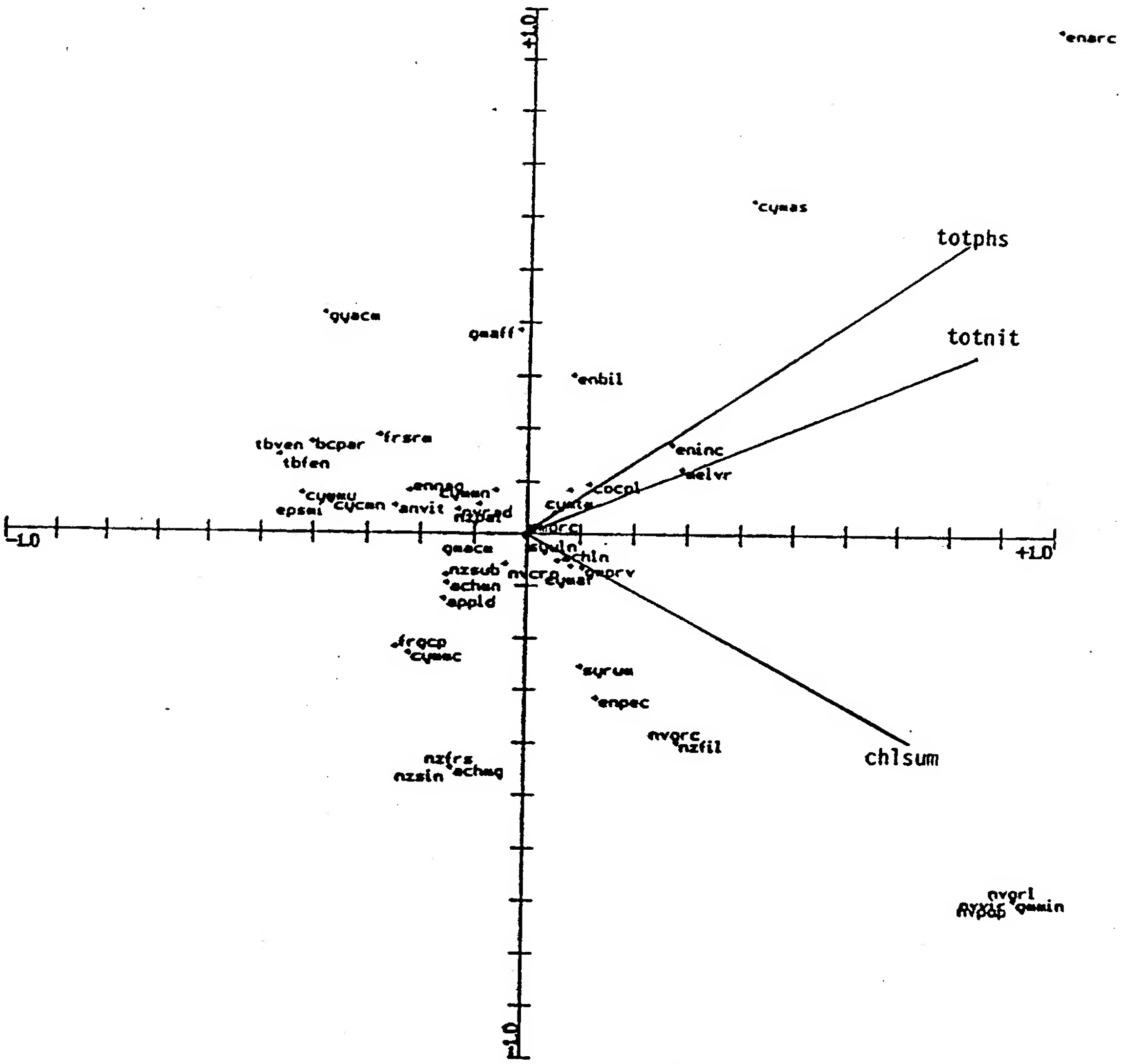

Figure 6. CCA biplot. Little River. Species/nutrients/chlorophyll
totphs = total phosphorous, totnit = total nitrogen, chlsum = chlorophyll

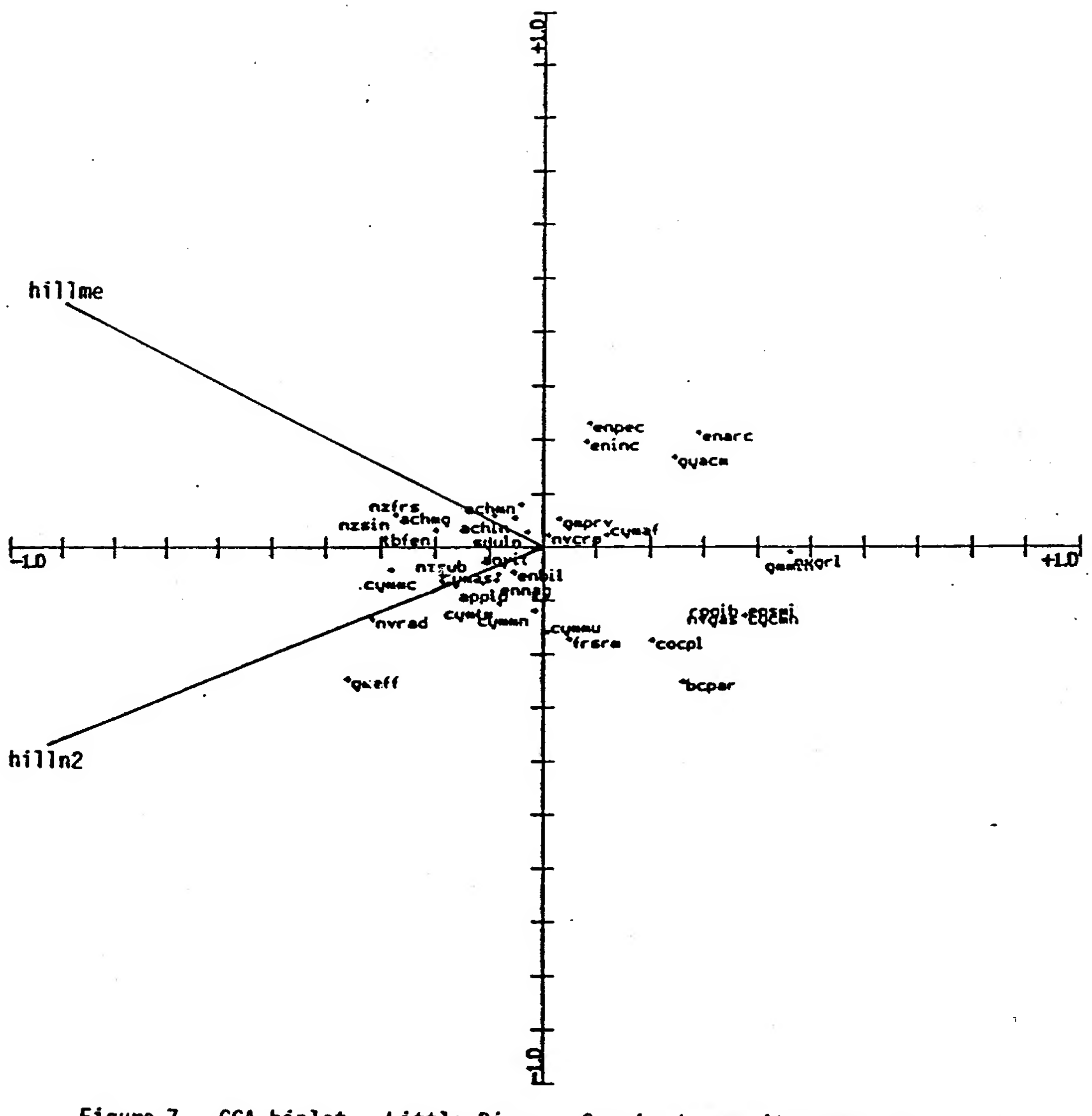

Figure 7. CCA biplot. Little River. Species/community measures.
hillme = Hills Modified Evenness, hilln2 = Hills N2 diversity index

A summary of the CCA output is contained in Table 12. Variable correlations are similar for the first axis which can be seen in the relatively high degree of collinearity in Figure 5. This association becomes much less pronounced on axis 2 as the three variables are more disjunct; however, on axis 3 there is an association between nitrogen and chlorophyll, although as seen in the low eigenvalue and relatively low species/environment correlation for this axis, this accounts for little of the variation in the data. For the measures of community structure, it can be seen that the two variables have a high degree of covariance with each one having approximately equal responsibility for data variance.

Table 12. Summary of CCA output - Little River

	Axis 1	Axis 2	Axis 3
Eigenvalues	0.311	0.225	0.086
Species/Environment corr.	0.885	0.843	0.656
Variable correlations:			
Total Nitrogen	0.743	0.290	0.275
Total Phosphorous	0.732	0.474	-0.009
Chlorophyll a	0.642	-0.327	-0.373

	Axis 1	Axis 2
Eigenvalues	0.327	0.139
Species/Environment corr.	0.862	0.824
Variable correlations:		
Hills N2	-0.799	-0.310
Modified Hill Evenness	-0.772	-0.367

c. Muddy Boggy System

Figures 8 and 9 contain the CCA biplot output from the Muddy Boggy System. Again, particular species can be seen to be associated along the nutrient and chlorophyll gradients; however, there is a clear species distribution gradient along two of the quadrants not occupied by any environmental gradient. This indicates that other factors have a significant influence on the species distribution. Similar species distributions can be seen along diversity and evenness gradients.

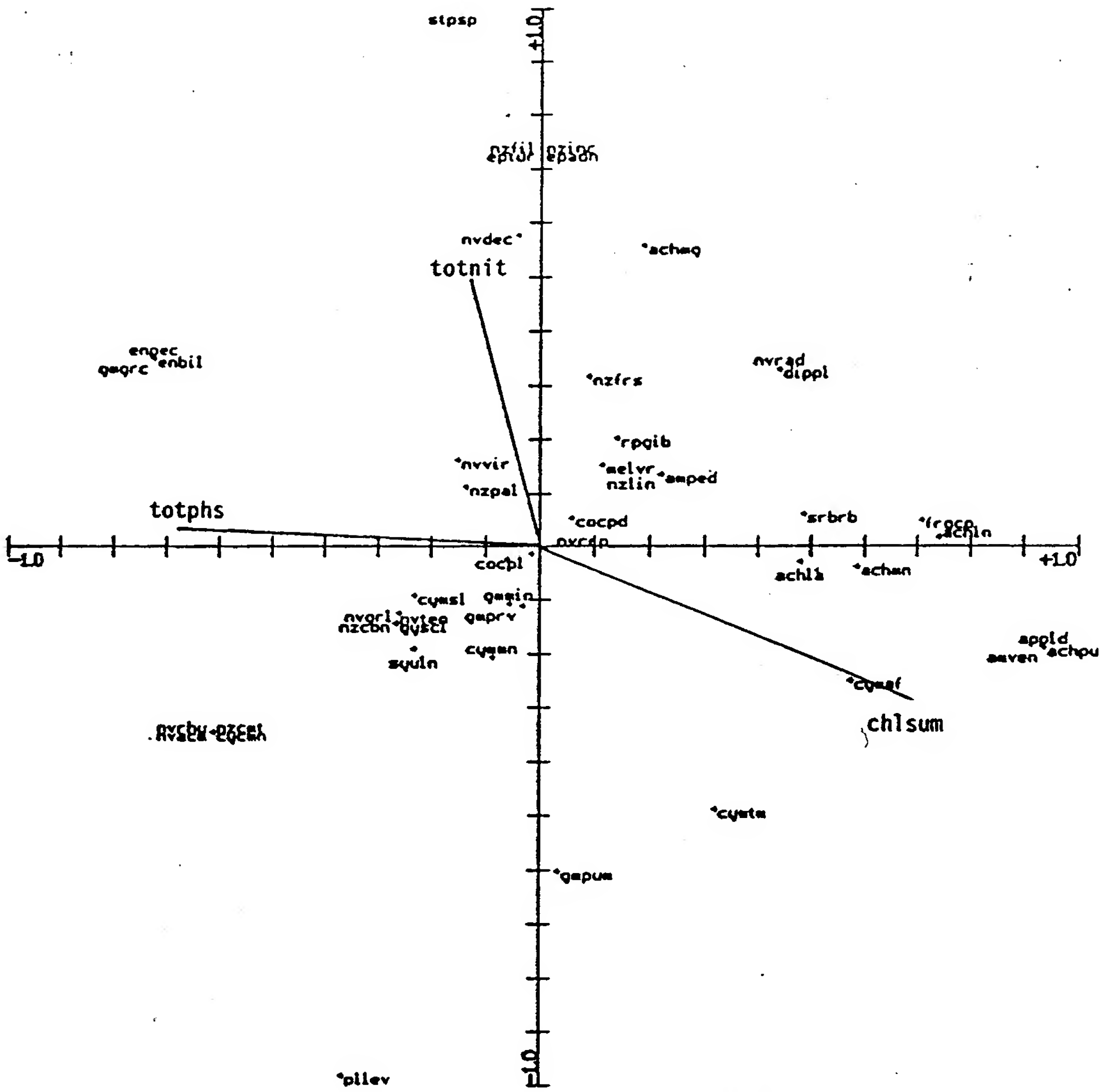

Figure 8. CCA biplot. Muddy Boggy. Species/nutrient/chlorophyll.
totphs = total phosphorous, totnit = total nitrogen, chlsum = chlorophyll

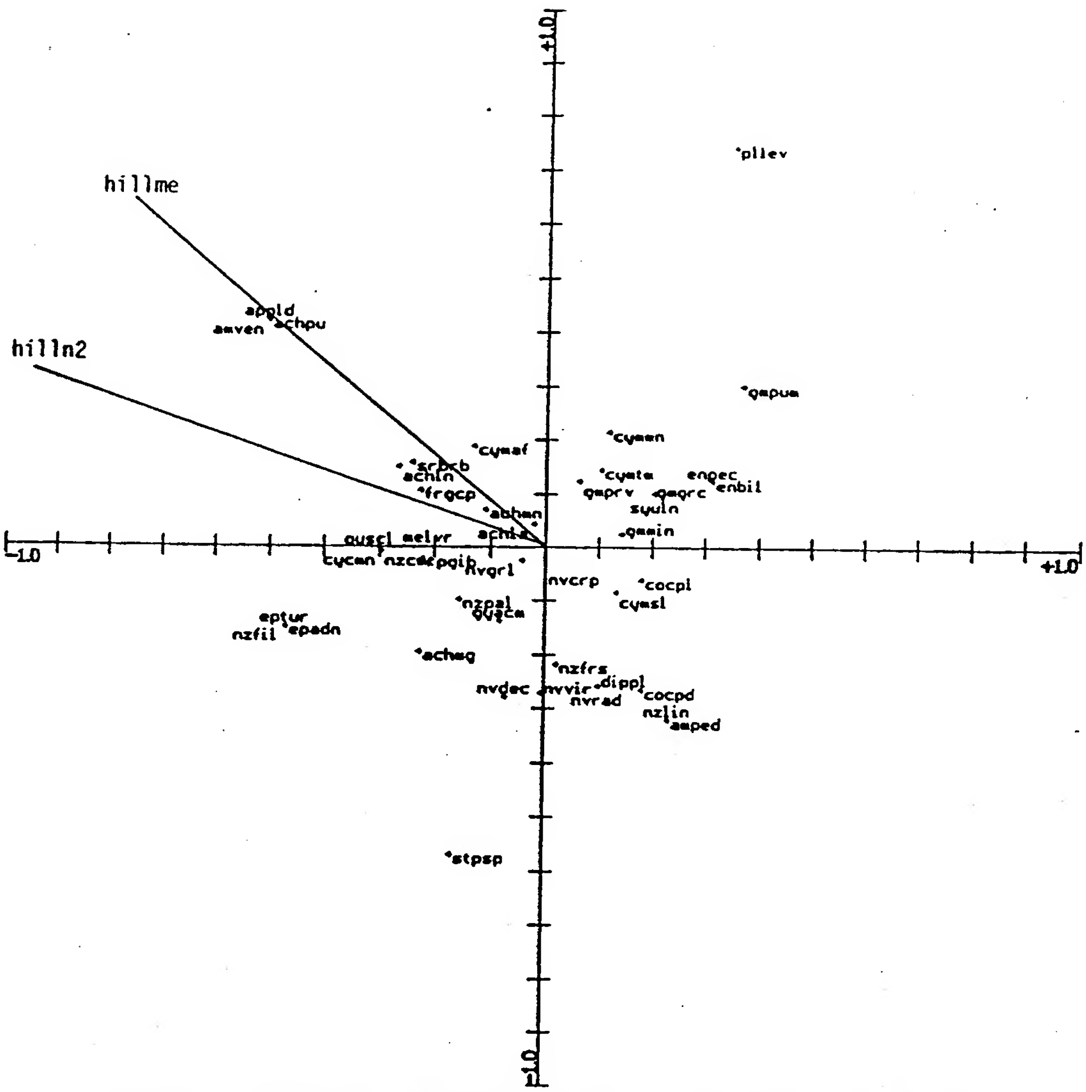

Figure 9. CCA biplot. Muddy Boggy System. Species/community measures.
hillme = Hills Modified Evenness, hilln2 = Hills N2 diversity index

A summary of the CCA output is contained in Table 13. The eigenvalue for axis 1 is relatively high resulting in a very high species/environment correlation. It can be seen that chlorophyll and phosphorous are primary determinants of this gradient as seen in their similar magnitudes; however, it is interesting that the signs of these two variables are opposite. It could be inferred from these data that at sites where phosphorous is high, chlorophyll is low, and vice versa. This defies common understanding but it helps to explain why there was no significant chlorophyll-phosphorous correlation in the Spearman Rank Correlation test. This anomaly could probably best be explained by the previous conclusion that neither phosphorous (nor nitrogen) are limiting nutrients in this study area. The fact that the two variables move in opposite directions; therefore, is probably an artifact of the test and not due to any cause-effect relationship. Axis 2 shows a similar trend; however, in this case nitrogen, rather than phosphorous, is more negatively associated with chlorophyll. On axis 3 the three variables coincide; however, the low eigenvalue indicates that this axis is relatively insignificant in explaining data variance. Given the unusual nature of this data and the obvious absence of an important environmental variable in this data, it is probably not prudent to make extensive associations between environmental gradients and individual species.

Table 13. Summary of CCA output - Muddy Boggy

	Axis 1	Axis 2	Axis 3
Eigenvalues	0.506	0.311	0.190
Species/Env. corr.	0.976	0.885	0.870
Variable correlation:			
Total Nitrogen	-0.114	0.423	0.758
Total Phosphorous	-0.661	0.033	0.640
Chlorophyll a	0.684	-0.254	0.569

	Axis 1	Axis 2
Eigenvalues	0.327	0.139
Species/Env. corr.	0.862	0.824
Variables corr.:		
Hills N2	-0.884	0.294
Modified Hill Evenness	-0.713	0.560

III. Inter-area Differences

Perhaps the most interesting output from CCA analysis concerns differences in species distributions and their association with environmental gradients between study areas.

Changes in chlorophyll were most closely associated with nitrogen and phosphorous in the Little River study area and somewhat less so in the Illinois River area. For the Muddy Boggy system it can be seen that the chlorophyll gradient ran in the opposite direction of nitrogen and phosphorous. As was mentioned in the previous section, no linear association was found between these parameters in the Muddy Boggy system and the CCA output clearly demonstrates that other factors are controlling the levels of chlorophyll.

Associations between nutrient gradients and species were more pronounced in the Illinois and Little River study areas than the Muddy Boggy system. A much stronger association was found between the chlorophyll gradient and species distribution in the Little River than the other two study areas. The fact that nutrient and community structure gradients move in opposite directions demonstrates that in each of the three study areas as nutrients increased, diversity and evenness decreased. This was more pronounced in the Illinois and Little River study areas and less so in the Muddy Boggy system. Figure 10 shows the association between nutrients and the measures of community structure in the three study areas. A similar analysis was done comparing community measures and chlorophyll (Figure 11). For the Illinois River and Little River, the chlorophyll gradient lies in the opposite direction of the community structure gradients, although this relationship is not strong, which is seen as the gradients move away from parallel. It is assumed that as chlorophyll increases beyond a certain point diversity decreases. The fact that these gradients are not exactly opposite indicates that this is not completely true in these data. This may occur as a result of the inclusion low chlorophyll values and the fact that the diversity decrease does not occur until a certain chlorophyll threshold is reached. Again, the data from the Muddy Boggy stands out as all three of these gradients run in the same direction. It is doubtful that this is a cause/effect relationship; therefore, this may be due to other factors such as inaccurate chlorophyll values or a preponderance of values below the threshold where decreases in diversity occur. This results in higher weights being give to low diversity values in the CCA output.

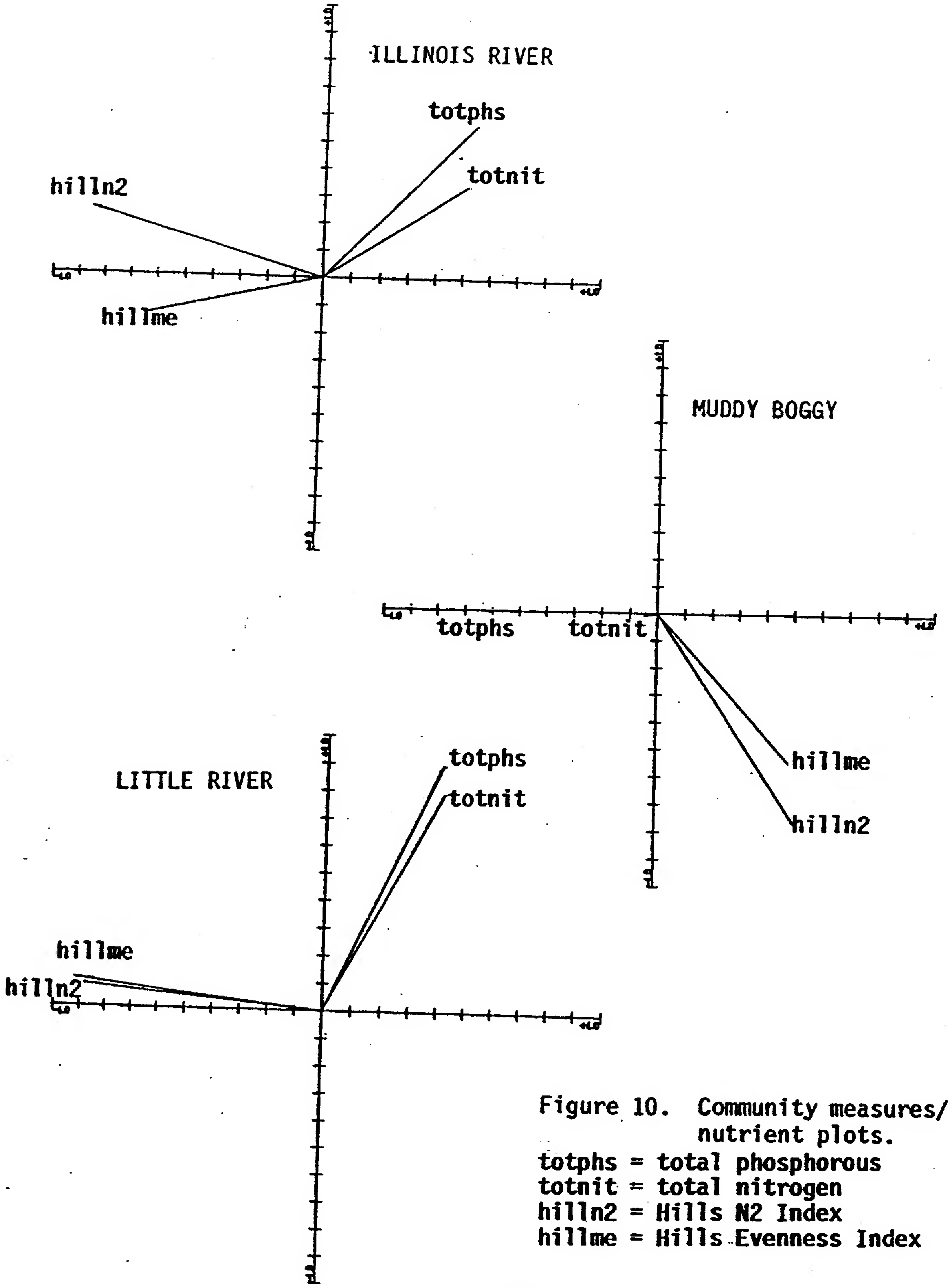

Figure 10. Community measures/
nutrient plots.
totphs = total phosphorous
totnit = total nitrogen
hilln2 = Hills N2 Index
hillme = Hills Evenness Index

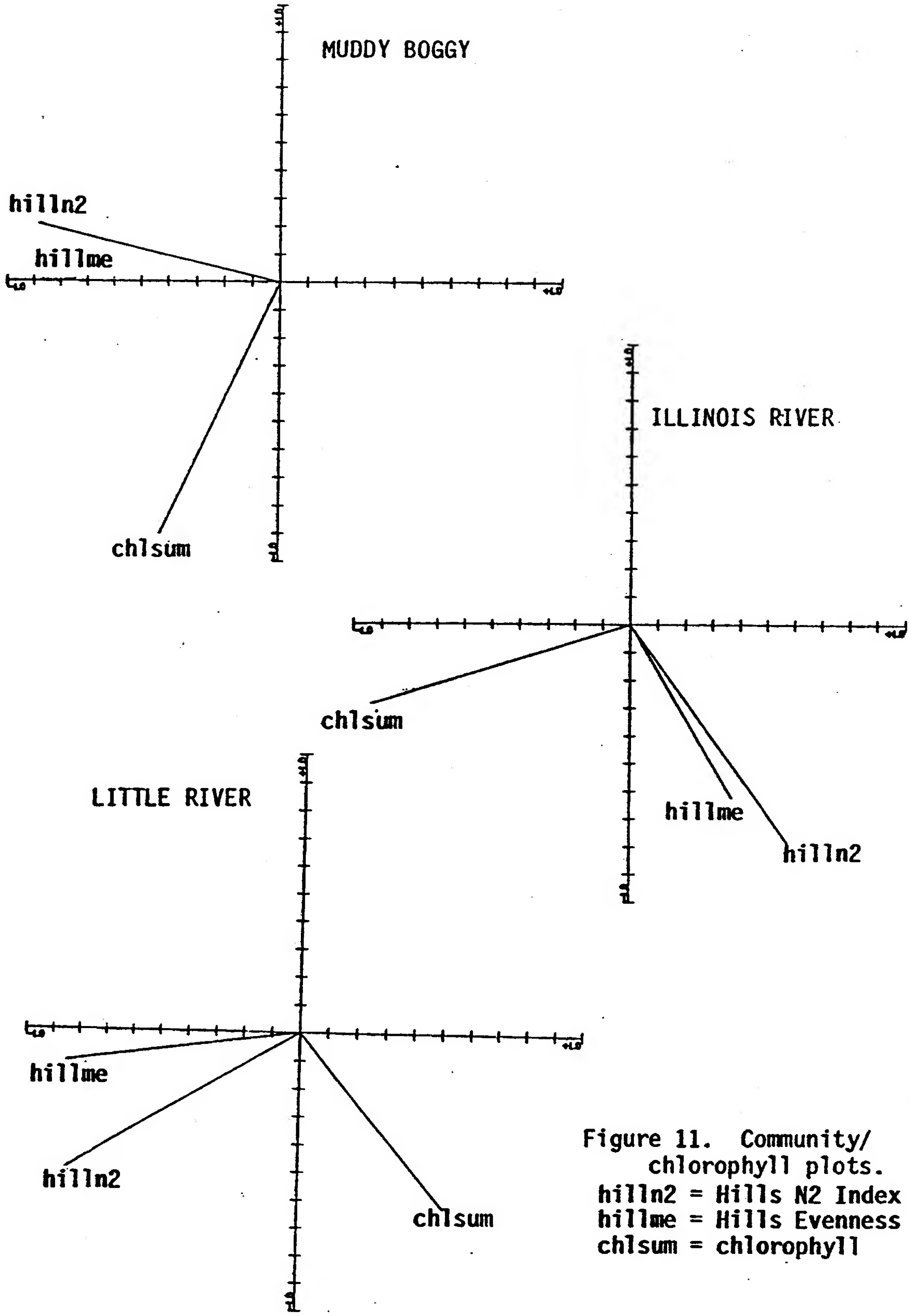

Figure 11. Community/
chlorophyll plots.
hilln2 = Hills N2 Index
hillme = Hills Evenness
chlsum = chlorophyll

Conclusions:

Several conclusions can be drawn from CCA analysis; however, the most important are that: 1) the three study areas are distinct based on species/environment interactions, 2) ecoregional differences area more pronounced in the summer, 3) the Muddy Boggy system is very different from the other two study areas and 4) certain species are closely related to environmental variables. All of these factors are helpful in establishing diatom RBPs for the following reasons:

1) The fact that the three areas are distinct indicates that statewide criteria can only be established for RBPs in terms of an ecoregional framework since ecoregional reference conditions will be different.

2) The timing of wide-scale collection should be planned for warmer months (unless the timing of an environmental insult is known to occur during another period).

3) Conclusion #3 strengthens the case for biological assessments as it demonstrates that nitrogen and phosphorous are not always the controlling factors in algal production.

4) The indicator value for species for which environmental indicator information cannot be found in the literature can be determined from their position along environmental gradients. The response of an individual species is obviously more important in a local setting; therefore, individual species should always be examined in terms of their place on locally derived environmental gradients.

CHAPTER 10

SUMMARY AND CONCLUSIONS

The purpose of this project was to synthesize rapid bioassessment protocols, using characteristics of the diatom community, by combining previously published information and data gathered from field research. As a result, three protocols were developed which use various quantitative and qualitative aspects of the diatom community and which provide different degrees of assessment. The degree of assessment is based upon the amount and detail of the information collected during individual field studies.

There have been numerous previous attempts to classify stream based upon the structure of the diatom community; however, most of these were based on a single measurement, such as a single diversity index, or were directed at very polluted areas. This research focused on a number of community measures (metrics) and on less polluted streams. Community measures were found to be most effective when conducted during the summer using artificial substrates. It was also shown that a 100 organism sample size was adequate to determine diatom community characteristics as opposed to a 250 organism sample size.

The three protocols which were developed, allow for stream assessment to be based both upon the level of knowledge required and on the financial and human resources of the assessment program. The screening protocol (#I) will allow for a very rapid assessment of stream quality when only fundamental (impaired/un-impaired) information is required. Protocol II & III assess streams based on their condition relative to that of demonstrably obtainable reference conditions within defined geographical regions. Analysis of chemical data and site/species interactions revealed that there were significant differences between the three study areas in terms of chemical quality and species composition and the response of diatom communities to levels.

It can be concluded from these data that diatom communities and ecological conditions are not homogeneous across the state and that geographically defined areas (ecoregions) have a combination of conditions which result in definite regional characteristics. The relatively simple environmental measures which were employed in this study were able to separate out ecoregional differences as well as differences in streams within ecoregions.

The assessment protocols should provide a useful tool for assessing stream quality; however, they will best employed when combined with fish and benthic macroinvertebrate analyses. This would allow for a more complete analysis of stream conditions. There are a number of factors which could potentially interfere with the results of any bioassessment; therefore, some caution should be taken before

a particular source of contamination is tagged as the cause of stream pollution. Whenever possible bioassessment should be combined with chemical analyses, although they are capable of producing adequate stream assessments in the absence of chemical data.

CHAPTER 11

RECOMMENDATIONS FOR ADDITIONAL RESEARCH

This research, as all others, would have been strengthened by the addition of more sample sites and more sampling dates. Particularly useful would have been multiple sampling dates within a single summer season.

If this type of assessment is to be extended, many additional sites will have to be sampled across Oklahoma. Western regions are very different geologically and climatologically from the eastern part of the state where this research was combined. From this research it would appear that twenty sites, if well selected, offer an adequate measure of a range of response across an ecoregion; however, this number may prove too small or large in other areas, especially when ecoregions cover wide latitudes or longitudes.

A special need is more emphasis on reference conditions. There is little institutional interest in non-polluted streams; however, it is obvious that reference-based assessment protocols must have the most accurate measure of practically obtainable conditions. In this regard several high quality streams should be sought out in each ecoregion.

The data from this project shows that streams receiving sewage treatment plant (STP) wastes responded similarly and were quite different from other streams. The degree and quality of sewage treatment varies greatly and it should be possible to establish reference conditions below the highest quality STP operations so that other plants could be judged on the basis of these reference conditions.

Although a number of taxonomic reference books are available, many are not easily accessible as they are written in German and are very expensive. Additionally, it was mentioned that these books tend to include all of the diatom flora of a given area and rarely focus on common species. A "Diatoms of Oklahoma" would be useful; however, a condensed version of the "Diatoms of the United States" which focuses only on the more common species would be equally helpful and would advance the cause of diatom-based stream assessments.

A long-term research goal research goal is the integration of diatom protocols with fish and benthic macroinvertebrate protocols into a single biological assessment of stream integrity.

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* denotes literature which was used in developing indicator values. These entries are not cited in the text.

Appendix 1. Dominant species and indicator values.

Species	# of times as co-dominant	Indicator Value
Achnanthes linearis	14	1
Achnanthes minutissima	13	1
Achnanthes lanceolata	5	2
Achnanthes exigua	1	3
Amphipleura pellucida	1	2
Anomoneis vitrea	7	1
Cocconeis placentula	49	3
Cocconeis pediculus	5	2
Cyclotella meneghiniana	1	2
Cymbella affinis	29	2
Cymbella aspera	1	2
Cymbella sinuata	1	2
Cymbella tumida	2	2
Diatoma vulgare	6	3
Epithemia adnata	2	1
Epithemia sorex	1	1
Eunotia bilunaris	5	1
Eunotia incisa	2	1
Eunotia pectinalis	1	1
Eunotia arcus	4	1
Eunotia pectinalis	1	1
Eunotia naegelii	1	1
Fragilaria capucina	8	1
Fragilaria fasciculata	1	2

Gomphonema subtile	2	1
Gomphonema utae	7	2
Gomphonema angustum	3	2
Gomphonema parvulum	28	3
Gomphonema olivaceum	2	3
Gomphonema minutum	11	2
Gomphonema clavatum	1	2
Gomphonema pumilum	1	2
Gomphonema affine	3	3
Gomphonema truncatum	1	1
Gomphonema clevei	2	2
Gomphonema acuminatum	3	1
Gomphonema gracile	3	1
Melosira varians	10	2
Meridion circulare	20	1
Navicula decussis	2	2
Navicula tenera	1	2
Navicula cryptocephala	11	1
Navicula graciloides	1	2
Nitzschia palea	2	3
Nitzschia dissipata	1	3
Nitzschia linearis	3	3
Nitzschia fonticola	1	2
Nitzschia frustulum	3	3
Rhopalodia gibba	3	1
Surirella brebissonii	1	2

<u>Syedra ulna</u>	56	3
<u>Tabellaria fenestrata</u>	1	1
<u>Tabellaria ventricosa</u>	1	1

Appendix 2. Site abbreviations and complete name.

Site #	Stream name
i01	Battle Branch
i02	Biddings Springs
i03	Black Fox Hollow
i04	Brushy Creek
i05	Cedar Hollow
i06	Cedar Hollow
i07	England Hollow
i08	Green Creek
i09	Little Saline Creek
i10	Luna Branch
i11	North Mining Camp Hollow
i12	Negro Jake Hollow
i13	Parkhill Branch
i14	Peacheater Creek
i15	Peavine Hollow
i16	Ross Branch
i17	Sager Creek
i18	Sager Creek
i19	Shell Creek
i20	Steely Hollow
i21	Sycamore Creek
i22	Tate Parris Hollow
i23	Tyner Creek
i24	Warren Branch Creek

L25	Beech Creek
L26	Lower Buffalo River
L27	Upper Buffalo River
L28	Caney Creek
L29	Cedar Creek
L30	Cucumber Creek
L31	Cypress Creek
L32	Cypress Creek
L33	Dry Creek
L34	East Fork Glover River
L35	Horsehead Creek
L36	Lukfata Creek
L37	Lukfata Creek
L38	Upper Terrapin Creek
L39	Mine Creek
L40	Mud Creek
L41	Pickens Creek
L42	Pine Creek
L43	Six mile Creek
L44	Upper Little River
L45	Upper Little River
L46	West Fork Glover River
L47	Wildhorse Creek
m48	Bois D'Arc Creek
m49	Chickasaw Creek
m50	Clear Boggy Creek

m51	Little Blue Creek
m52	Lower Mill Creek
m53	Upper Mill Creek
m54	Mineral Bayou Creek
m55	North Boggy Creek
m56	Pennington Creek
m57	Upper Sandy Creek
m58	Spring Creek
m59	Sugar Creek
m60	Sulphur Creek
m61	Upper Blue River
m62	Lower Whitegrass Creek
m63	Upper Whitegrass Creek

Appendix 3. Species abbreviations and complete name.

Species name	Abbreviation
Achnanthes linearis	achln
Achnanthes marginulata	achmg
Achnanthes minutissima	achmn
Achnanthes pusilla	achpu
Achnanthes lanceolata	achla
Amphipleura pellucida	appld
Amphora pediculus	amped
Anomoneis vitrea	anvit
Bacillaria paradoxa	bcpar
Cocconeis pediculus	cocpd
Cocconeis placentula	cocpl
Cymbella affinis	cymaf
Cymbella mexicana	cymex
Cymbella microcephala	cymmc
Cymbella muelleri	cymmu
Cymbella tumida	cymtm
Diatoma vulgare	dtvul
Diploneis puella	dippl
Epithemia adnata	edadn
Epithemia turgida	eptur
Eunotia arcus	enarc
Eunotia bilunaris	enbil
Eunotia incisa	eninc
Eunotia naeglii	ennag

Eunotia pectinalis	enpec
Fragilaria capucina	frgcp
Fragilaria fasciculata	frgfs
Frustulia rhomboides	frsrm
Gomphonema acuminatum	gmacm
Gomphonema affine	gmaff
Gomphonema augustum	gmaug
Gomphonema clavatum	gmclv
Gomphonema gracilis	gmgrc
Gomphonema graciloides	gmgrl
Gomphonema minutum	gmmin
Gomphonema parvulum	gmprv
Gomphonema pumilum	gmpum
Gomphonema subtile	gmsub
Gomphonema truncatum	gmtrn
Gomphonema utae	gmute
Gyrosigma accuminatum	gyacm
Gyrosigma scalproides	gyscl
Melosira varians	melvr
Meridion circulare	mrcir
Navicula accuminatum	nvacm
Navicula cryptocephala	nvcrp
Navicula desussis	nvdec
Navicula graciloides	nvgrc
Navicula mensiculus	nvmen
Navicula pupula	nvpup

<u>Navicula</u> <u>radiosa</u>	nvrad
<u>Navicula</u> <u>rhyncocephala</u>	nvrhy
<u>Navicula</u> <u>tripunctata</u>	nvtrp
<u>Navicula</u> <u>viridula</u>	nvvir
<u>Nitzschia</u> <u>comutata</u>	nzcom
<u>Nitzschia</u> <u>constricta</u>	nzcon
<u>Nitzschia</u> <u>filiformis</u>	nzfil
<u>Nitzschia</u> <u>frustulum</u>	nzfrs
<u>Nitzschia</u> <u>linearis</u>	nzlin
<u>Nitzschia</u> <u>palea</u>	nzpal
<u>Nitzschia</u> <u>sinuata</u>	nzsin
<u>Nitzschia</u> <u>subacicularis</u>	nzsub
<u>Pleurosigma</u> <u>laevis</u>	pllev
<u>Stephanodiscus</u> sp.	stpsp
<u>Synedra</u> <u>rumpens</u>	syrum
<u>Synedra</u> <u>ulna</u>	syuln
<u>Tabellaria</u> <u>fenestrata</u>	tbfen
<u>Tabellaria</u> <u>ventricosa</u>	tbven

Appendix 4. Community Measure Indices

1. **Margalef Richness** = $\frac{S - 1}{\ln(n)}$

where: S = total number of taxa
n = number of individuals observed

2. **Mehinick Richness** = $\frac{S}{\sqrt{n}}$

where: S = total number of taxa
n = number of individuals observed

3. **Simpson Diversity Index** $(\lambda) = \sum_{i=1}^{s} p_i^2$

where: p_i is the proportional abundance of the *i*th species given by:

$$_i = \frac{n_i}{N}, \quad i = 1,2,3,\ldots,S$$

where: n_i is the number of individuals of the *i*th species and N is the known total number of individuals for all S species in the population.

4. **Shannon Diversity Index** $(H') = \sum_{i=1}^{s^*} (p_i \ln p_i)$

where: H' is the average uncertainty per species in an infinite community made up of S^* species with known proportional abundances p_1, p_2, p_3, ..., p_s^*. S^* and p_i's are population parameters and, in practice, H' is estimated as:

$$H' = -\sum_{i=1}^{s} \left[\left(\frac{n_i}{n}\right) \ln \left(\frac{n_i}{n}\right) \right]$$

5. **Hills N0** = S

where: S = is the total number of taxa observed

6. **Hills N1** = $e^{H'}$

where: H' = Shannon's Diversity Index

7. **Hills N2** = $1/\lambda$

where: λ = Simpson's Diversity Index

8. **Simpson Evenness** = $\dfrac{H'}{\ln(S)} = \dfrac{\ln(N1)}{\ln(N0)}$

where: H' = Shannon's Diversity Index
S = Number of taxa observed
N1 = Hills N1
N0 = Hills N0

9. **Sheldon Evenness** = $\dfrac{e^{H'}}{S} = \dfrac{N1}{N0}$

where: H' = Shannon's Diversity Index
S = Number of taxa observed
N1 = Hills N1
N0 = Hills N0

10. **Heip Evenness** = $\dfrac{e^{H'} - 1}{S - 1} = \dfrac{N1 - 1}{N0 - 1}$

where: H' = Shannon's Diversity Index
S = Number of taxa observed
N1 = Hills N1
N0 = Hills N0